Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

Microbiology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers related to the research across the whole spectrum of the subject. These including bacteriology, virology, mycology and parasitology. In addition, the journal promotes research on the impact of living organisms on their environment with emphasis on subjects such a resource, depletion, pollution, biodiversity, ecosystem…..etc

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.10 (1)pp. 77- 84 (2018)*

Egypt. Acad. J. Biolog. Sci., 10(1): 77-84 (2018)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
www.eajbs.eg.net

Relationship Between Storage Periods and microorganisms (Bacteria, Fungi and Yeasts) In Honey

Ali, M. A. M.[1]; Rawdaa R. Khalil[1] and H. M. Ramadan[2]

1- Plant protection Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
2- Plant protection Department, Faculty of Agriculture, Tanta University, Egypt

ARTICLE INFO

Article History
Received:17/5/2018
Accepted:28/6/2018

Keywords:
Honey
Storage
Microorganisms
Bacteria
Fungi
Yeasts

ABSTRACT

The relationship between storage periods and microorganisms (bacteria, fungi and yeasts) in honey samples was evaluated. Ripe honey was collected from honey bee colonies fed on natural flowers available in the study area in addition plain sugar syrup (1:1). The population was 1.40, 1.80, 2.53, 3.47 and 4.90 colony/sample for bacteria; 0.30, 0.50, 0.90 1.60 and 2,20 colony/sample for fungi and 2.70, 3.10, 3.90, 4.50 and 7.20 colony/sample for yeasts in honey samples stored at zero, 3, 6, 9 and 12 months, respectively. According to isolation and identification procedures of microorganisms in tested honey samples, three bacteria types *Bacillus brevis*, *Bacillus cereus* and *Clostridium botulism*, four fungi types *Aspergillus apis*, *Aspergillus niger*, *Cladosporium* sp. and *Penicillium* sp. and three yeasts types *Debaromyces* sp., *Lipomyces* sp. and *Saccharomyces* sp. were determined according to cultural, morphological and physiological characters. The data also summarized that, fungi were the least population when compared with bacteria and yeasts. *Clostridium botulism bacterium* was the most frequency (%) compared with other bacteria types. *A. apis* fungus was the most frequency (%) compared with other fungi types and *Lipomyces* sp. yeast was the most frequency, meanwhile the yeast *Saccharomyces* sp. was the least frequency. The data also summarized that, the population and frequency (%) of microorganisms increased as increasing the storage period.

INTRODUCTION

Honey is a product with minimal types and levels of microbes. Microbes of concern in post-harvest handling are those that are commonly found in honey (i.e., yeasts and spore-forming bacteria), those that indicate the sanitary or commercial quality of honey (i.e. coliforms and yeasts), and those that under certain conditions could cause human illness (Snowdon and Cliver, 1996). It contains fewer microorganisms than other neutral-foods. Microorganisms present in honey are those that can withstand the concentrated sugar, acidity and other anti-microbial components of honey. Conventional microbiology and PCR based studies reported several species of cultivable and non-cultivable bacteria, yeasts and filamentous fungi associated with honey of different geographical and botanical origin (Olivieri *et al.*, 2012, Sinacori *et al.*, 2014 and Rawdaa-Khalil, 2018).

There are some sources of microbial contamination in honey, the primary sources of microbial contamination are the nectar, pollen, the digestive-tracts of honey bees, dust, dirt, earth, and air, the other sources of microbial contamination in honey are post-harvest sources include dust, wind, containers, equipment and human (Olaitan *et al*., 2007 and Kim, *et al*., 2011). These microbes could be placed in three categories: microorganisms that are commonly found in honey (certain strains of yeast and spore-forming bacteria) (Aurongzeb and Azim, 2011); microorganisms that indicate sanitary or commercial quality (coliforms or yeasts) (Whadan, 1998); and microorganisms that infer certain conditions (e.g. germination and growth in a non-heat-treated food product) (Sinacori *et al*., 2014).Members of genera *Bacillus*, *Clostridium* and *Micrococcus* are common in air and dust and they can easily enter into honey (Rawdaa-Kalil, 2018).Sadik and Ali (2012) found that the numbers of aerobic mesophilic bacteria, moulds and yeasts were less than 105 cfu/g for all 91 samples in honey samples from different plant sources and regions of kingdom of Saudi Arabia. They also found that, total coliforms, *Salmonella* spp., *Shigella* spp, *E. coli.* and *Clostridium* sulfite-reducers were not detected but *Bacillus licheniformis*, *Bacillus wakoensis*, *Bacillus subtilis*, *Bacillus atrophaeus*, *Bacillus sonorrensis*, *Bacillus spizizenii*, *Bacillus vallismortis*, *Bacillus sonorensis*, *Bacillus alcalophilus*, *Bacillus murimartini*, *Bacillus horti* and *Aspergillus niger* were detected. *Paenibacillus larvae larvae* were not detected in all honeys samples. Estevinho *et al*. (2013) found that all organic honey samples showed low microbiological counts (yeast, moulds, and aerobic mesophiles), with negative results in respect to faecal coliforms, *Salmonella*, and sulphite-reducing clostridia. Rawdaa- Kalil (2018) found that, no bacteria, fungi and yeasts were detected in extracted honey at zero time, it was detected after 6 months, It also summarized that *Clostridium botulism* was the most frequency compared with *B. brevis* and *B. cereus* and the fungus *A. apis* was the most frequency compared with other fungi types.

On the other hand, high osmolarity, acidity and the presence of hydrogen peroxide in natural honey make the growth of microorganism difficult (Whadan, 1998 and Aurongzeb and Azim, 2011).Presence of high sugar concentration, low pH, hydrogen peroxide, antioxidants, polyphenols, phenolic acids, and bee peptides contributes to its anti-bacterial activity (Wajiha Gul *et al*., 2015). The antioxidant activity of honey varies depending upon the floral source, climatic conditions, geographical origin, processing and storage (Belitz *et al*., 2009). Since honey has antimicrobial properties that discourage the growth or persistence of many microorganisms, it has been used as a medicine since ancient times in many cultures. Honey vary according to their plant origin and the conditions of their production (Bogdanov, 1997) and it shows antibacterial activity against the growth of *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Radwan, *et al*., 1984; Efem, 1993 and Agbagwa and Frank-Peterside 2010).

The objective of the current research is to study the relationship between the storage period and population as well as the types and frequency (%) of microorganisms in stored honey.

MATERIALS AND METHODS

The experimental honey bee colonies were fed on natural flowers which available in the study area in addition plain sugar syrup (1:1), they were fed weekly on 400 ml of sugar syrup (1 sugar: 1 water)/ colony).

The ripe honey was extracted from experimental honeybee colonies at end of the experiment and honey samples were collected in 250 sterilized glass jars, and then transferred to Bacterial Plant Diseases laboratory, Plant Diseases Department, Faculty of Agriculture, Ain Shams University for isolation, purification and identification of microorganisms (bacteria,

fungi and yeasts).The samples were used immediately (zero time) or stored at 3, 6, 9 and 12 months at room temperate to study the relationship between storage periods and the population of microorganisms (bacteria, fungi and yeasts) in stored honey.

Isolation of Microorganisms in Honey Samples:

Nutrient agar (NA), water agar (WA) and nutrient yeast extract dextrose agar (NYDA) media were applied to study isolation and population of bacteria, fungi and yeasts, respectively. Honey suspension was prepared by adding 10 g of honey to flask contained 90 ml of sterilized distilled water and was shaken at 1000 rpm for 2hr. One ml of the prepared solutions was added onto sterilized petri dish and about 20 ml of previously method specific agar media (45-50^{0}C) were added to it (Mehrotra *et al*., 1996; Barnett and Hunter 1987 and Fahy and Persley 1983). Four plates were used as replicates for each particular treatment. After gentle rotation, inoculated plates were incubated at 28^{0}C for 2-5 days. Number of bacteria, fungi and yeasts colonies were recorded per sample to calculate their population. Single colonies were picked up and transferred to another slant media. Obtained bacteria, fungi and yeasts isolates were kept in a refrigerator for further studies (Rawdaa-Khalil, 2018).

Identification of Microorganism in Stored Honey Samples:

Selected colonies of bacteria, fungi and yeasts were transferred to sterilized Petri dishes contained the specific medium for purification by steaked plate technique and incubated at 28^{0}C for 2.5 days. According to cultural, morphological and physiological characters, selected bacteria (Schaad, 1980 and Bochner, 1991), fungi (Barnett and Hunter, 1987); and yeasts (Kreger-Van Rij, 1984; Odds, 1988 and Barnett *et al*., 2000) were identification in the microbiology center, Faculty of Science, Al-Azhar University, Cairo, Egypt.

Statistical Analysis:

Obtained data were statistically analyzed by using a randomized complete block design in factorial arrangement according to Sndecor and Cocheran (1990). For separation between means, least significant difference LSD, (at 5% probability) was applied.

RESULTS

Population of Microorganisms (bacteria, fungi and yeasts) in Stored Honey Samples:

As shown in Table (1) the population of bacteria was 1.40, 1.80, 2.53, 3.47 and 4.90 colony/sample; 0.30, 0.50, 0.90 1.60 and 2,20 colony/sample for fungi and 2.70, 3.10, 3.90, 4.50 and 7.20 colony/sample for yeasts in honey samples stored at zero, 3, 6, 9 and 12 months, respectively. The data indicated that, the population of microorganisms increased as increasing the storage period, where honey samples stored at 12 months had the highest population of bacteria, fungi and yeasts followed by 9 months. Meanwhile, samples stored at zero, 3 and 6 months had the least microorganisms count with significant difference between all the storage periods.

Table 1: Mean population count of microorganisms (bacteria, fungi and yeasts) in honey samples stored at different storage periods under room temperature condition.

Storage period (month)	Mean colonies count /honey sample of different storage periods		
	Bacteria	Fungi	Yeasts
0	1.40± 0.66e	0.30± 0.63d	2.70± 0.58e
3	1.80± 0.08d	0.50± 0.52d	3.10± 0.33d
6	2.53± 0.09c	0.90 0.62c	3.90± 0.70c
9	3.47± 0.99b	1.60± 0.72b	4.50± 0.65b
12	4.90± 1.01a	2.20± 0.81a	7.20±0.77a
L.S.D.	0.384	0.244	0.282

The data also summarized that fungi were the least population as compared with bacteria and yeasts, where their population ranged from 0.30 to 2.20 colony/sample,

while it ranged between 1.40 to 4.90 colony/sample for bacteria, meanwhile, the population of yeasts registered the highest population counts ranged from 2.70 to 7.20 colony/sample (Table, 1).

Type and Frequency (%) of Bacteria:

The frequency (%) of *B. brevis*was 0.6, 1.4, 2.80, 7.9 and 15.7%, *B. cereus* was 0.1, 0.9, 2.1, 3.2 and 5.0% and *C. botulism* was 0.6, 1.7, 3.0, 8.7 and 17.3% in honey samples stored at zero, 3, 6, 9 and 12 months, respectively (Fig. 1).

The data also show that, the frequency (%) of bacteria increased as increasing the storage period. It also summarized that, *Clostridium botulism bacterium* was the most frequency (%) compared with other bacteria types. Their percentages of frequency ranged 0.6 – 17.3, 0.6 – 15.7 and 0.1 – 5.0% for *C. botulism*, *B. brevis* and *B. cereus*, respectively (Fig.1).

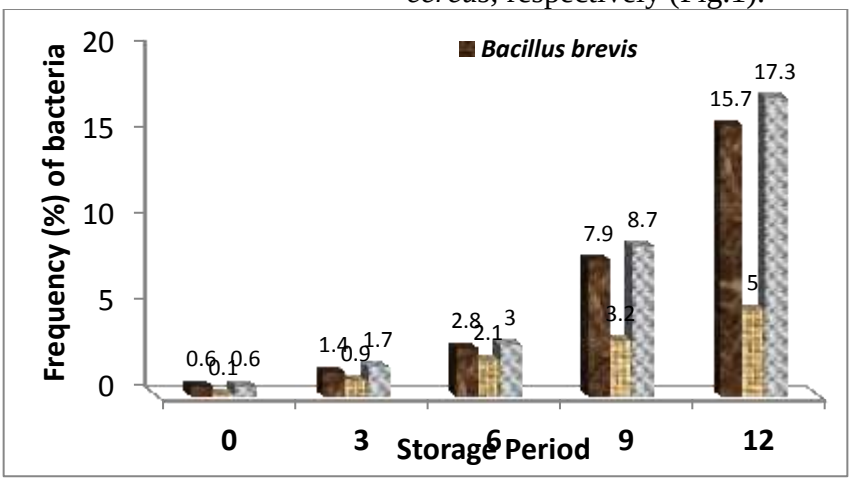

Fig. 1: Relationship between storage periods and frequency (%) of bacteria in honey samples.

Type and Frequency (%) of Fungi:

The percentage of frequency of *A. apis* fungus was 0.5, 1.3, 2.9, 8.5 and 16.3%; 0.2, 1.0, 2.8, 6.5 and 12.4% for *A. niger*; 0.0, 0.0, 0.2, 0.9 and 1.6 % for *Cladosporium* sp. and 0.0, 0.0, 0.7, 2.1 and 4.3 for *Penicillium* sp. after 0, 3, 6, 9 and 12 months, respectively (Fig. 2).

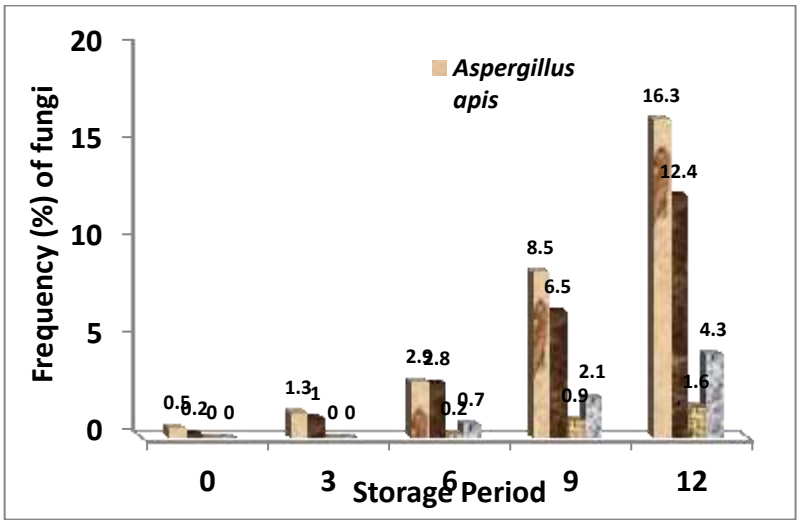

Fig. 2: Relationship between storage periods and frequency (%) of fungi in honey samples.

The data showed that, the frequency (%) of fungi increased as increasing the storage period. Also, *A. apis* fungus was the most frequency compared with other fungi types, where its frequency ranged from 0.5 to 16.3%, followed by *A. niger* 0.2 to 12.4%, meanwhile, *Cladosporium* sp. and *Penicillium* sp. were the least frequency ranged from 0.0 to 1.6 and 0.0 to 4.3, respectively (Fig. 2).

Type and Frequency (%) of Yeast:

Data illustrated in Fig. (3) showed that, the percentage of frequency (%) was 0.2, 1.2, 3.4, 7.0 and 13.2%for *Debaromyces* sp.; 0.4, 1.3, 3.1, 6.9 and 15.4% for *Lipomyces* sp. and 0.0, 0.2, 1.1, 3.0 and 5.4% for*Saccharomyces* sp. after 0, 3, 6, 9 and 12 months, respectively.

The data indicated that, the frequency of fungi increased as increasing the storage period. It also summarized that*Lipomyces* sp. was the most frequency ranged from 0.4 to 15.4%, meanwhile the yeast *Saccharomyces* sp. was the least frequency 0.0 to 5.4% (Fig. 3).

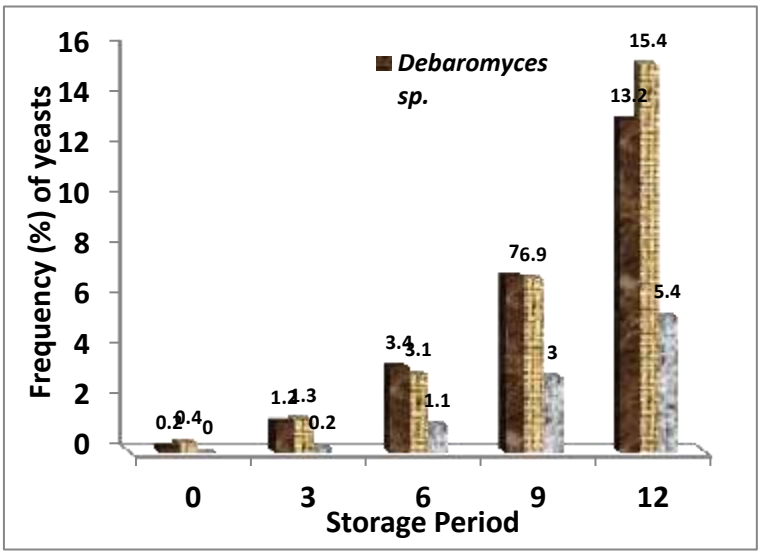

Fig. 3: Relationship between storage periods and frequency (%) of yeasts in honey samples.

DISCUSSION

According to isolation and identification procedures of microorganisms in tested honey samples, three bacteria types *Bacillus brevis*, *Bacillus cereus* and *Clostridium botulism*, four fungi types *Aspergillus apis*, *Aspergillus niger*, *Cladosporium* sp. and *Penicillium* sp. and three yeasts types *Debaromyces* sp., *Lipomyces* sp. and *Saccharomyces* sp. were determined according to cultural, morphological and physiological characters. It also summarized that, fungi were the least population when compared with bacteria and yeasts. *Clostridium botulism bacterium* was the most frequency (%) compared with other bacteria types, *A. apis* fungus was the most frequency compared with other fungi types and *Lipomyces* sp. yeast was the most frequency. The data also summarized that, the population and frequency (%) of microorganisms increased as increasing the storage period.

The obtained data is in agreement with data by Dumen *et al*., 2013; Estevinho *et al*., 2013; Oliveira *et al*., 2013; Tasneem and Aruna, 2013; Belas *et al*., 2014; Musa *et al*., 2014) whom found that honey samples showed low microbiological counts (yeast, moulds, and aerobic mesophiles), with negative results in respect to faecal coliforms and*Salmonella* and Monte *et al*. (2013) who found that the fungal count ranged from 1.77 to 2.26 CFU/g log 10 in any of the honey samples.It also with agreement with results obtained byRawdaa-Khalil(2018) who isolated bacteria, fungi and yeasts from

honey samples and found that fungi were the least population when compared with bacteria and yeasts and not detect either *Salmonella* or *E. colli* intested honey samples.

It also is in disagreement with Adjlane *et al*. (2014) who found that Total coliforms and *Clostridium* were not detected in honey sample collected from Algeria and data from Gul *et al*. (2015) who found a list of microorganisms in honey samples collected from Karachi, Pakistan included bacteria *E-coli* and *Pseudomonas* in honey samples.

ACKNOWLEDGMENT

The authors have to thank Prof. Dr Nagy Yasin Abd-Elgfar, Plant Diseases Department, Faculty of Agriculture, Ain Shams University for allowing us to conduct part of this study in Bacterial Plant Diseases laboratory. Our thanks also go to Microbiology Center, Faculty of Science, Al-Azhar University, Cairo, Egypt for microorganisms identification.

REFERENCES

Adjlane, N.; N. Haddad; K. L. Ameur; S. Kesraoui and D. Moussaoui (2014). Physicochemical and microbiological characteristics of some samples of honey produced by beekeepers in Algeria. Acta Technologica Agriculturae; 17(1):1-5. 27 (AN: 20143327792).

Agbagwa, O. E. and N. Frank-Peterside (2010). Effect of raw commercial honeys from Nigeria on selected pathogenic bacteria. African Journal of Microbiology Research; 4(16):1801-1803 (AN: 20113228091).

Aurongzeb, M. and M. K. Azim (2011). Antimicrobial properties of natural honey: a review of literature. Pak. J. Biochem. Mol. Biol.; 44: 118-124.

Barnett, H. L. and B. B. Hunter (1987). Illustrated Genera of imperfect Fungi. Burgress Co., Minneapolis, Minnesota, USA, 241pp.

Barnett, J. A.; R. W. Payne and D. Yarrow (2000). Yeasts characterization and identification. Cambridge University Press, Cambridge, UK, 351 pp.

Belas, A.; C. Almeida; A. F. Epifanio; B. Carrapico; Y. Vaz and B. S. Braz (2014). Quality of national honey. Revista Portuguesa de Ciencias Veterinarias; 109(591/592):112-119 (AN: 20153348941).

Belitz, H. D.; W. Grosch and P. Schieberle (2009). Food Chemistry 4th ed.; 886-890.

Bochner, B. R. (1991). Identification of over 500 Gram-negative species by a single test panel. American Clinical Laboratory, 14 pp.

Bogdanov, S. (1997). Nature and origin of the antibacterial substances in honey. Lebensmittal Wissenschaft and Technology 30(7): 748-753.

Dumen, E.; H. Akkaya; G. M. Oz and F. H. Sezgin (2013). Microbiological and parasitological quality of honey produced in Istanbul. Turkish Journal of Veterinary & Animal Sciences; 37(5):602-607 (AN: 20133359447).

Efem, S. E. (1993). Clinicopathological features of untreated fibrous hamartoma of infancy. Clin Pathol.; 46: 522-524.

Estevinho, M. L.; M. P. Vazquez-Tato; J. A. Seijas and X. Feas (2013). Palynological, physicochemical, and microbiological attributes of organic lavender (*Lavandula stoechas*) honey from Portugal. Acta Alimentaria (Budapest); 42(1):36-44 (AN: 20133124342).

Fahy, A. C. and G. T. Persley (1983). Plant Bacteria Diseases; A Diagnostic Guide. Academic Press, New York, 393 pp.

Gul, W.; N. Farooq; U. Khan, F. Rehan and D. Anees (2015). Honey: A. Nectarous anti-infective agent. Word Journal of Pharmacy and Pharmaceutical Sciences. 4 (4): 208-215 (ISSN 2278 – 4357).

Rawdaa-Khalil, R. (2018). Survey and identification of microorganisms in some honeybee products. M.Sc.

Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Kim, S. A.; S. W. Oh; Y. M. Lee; J. Y. Imm; I. G. Hwang; D. H. Kang and M. S. Rhee (2011). Microbial contamination of food products consumed by infants and babies in Korea. Lett. Appl. Microbiol.; 53: 532-538.

Kreger-Van Rij, N. I. W. (1984). The yeasts: A taxonomic study, 3rd Ed., Elsevier Science Publishers, Amsterdam, Netherland, 301 pp.

Mehrotra, N. K.; N. Sharma; R. Ghosh and M. Nigam (1996). Biological control of green and mould disease of citrus fruit by yeasts. Indian Phytopathol. 49: 350-354.

Musa, M. Y.; A. E. Elfaki and S. E. A. Mohammed (2014). Microbiological characterization and physicochemical properties of Sudanese honeys. British Microbiology Research Journal; 4(6):715-722 (AN: 20143123000).

Odds, F. C. (1988). Candida and Candidosis, 2rd Ed., Bailliere Tindall, London, UK, 189 pp.

Olaitan, P. B.; O. E. Adeleke and I. O. Ola (2007). Honey: a reservoir for microorganisms and inhibitory agent for microbes. Afr. Health Sci., 2007; 7: 159-165.

Oliveira, K. A.; M. L. S. de Ribeiro; G. V. de Oliveira (2013). Microbiological characterization, physical-chemical and microscopic honey bee straw (*Scaptotrigona depilis*) and Jatai (*Tetragonisca angustula*). Revista Brasileira de Produtos Agroindustriais; 2013. 15(3):239-248 (AN: 20133309082).

Olivieria, C.; I. Marota; F. Rollo and S. Luciani (2012). Tracking plant, fungal, and bacterial DNA in honey specimens. J. Forensic Sci., 2012; 57: 222-227.

Radwan, S.; A. El-Essawy and M. M. Sarhan (1984). Experimental evidence for the occurrence in honey of specific substances active against microorganisms. Zentral Mikrobiol 139: 249 – 55.

Sadik, M. W. and M. A. M. Ali (2012): Survey and identification of microorganisms in Bee Honey samples collected from different plant sources and regions in Saudi Arabia. Global Advanced Research Journal of Microbiology (ISSN: 2315-5116), 1(8): 126-134.

Schaad, N. W. (1980). Laboratory guide for identification of plant pathogenic bacteria. The American Psychopathological Society, St. Paul, Minnesota, USA, 72 pp.

Sinacori, M.; N. Francesca; A. Alfonzo; M. Cruciata; C. Sannino; L. Settanni and G. Moschetti (2014). Cultivable microorganisms associated with honeys of different geographical and botanical origin. Food Microbiology; 38:284-294 (AN: 20143057031).

Sndecor, G. W. and W. G. Cocheran (1990). Statistical Methods, 7th Ed., Iowa State University Press, Ames, Iowa, USA, 507 pp.

Snowdon, J. A. and D. O. Cliver (1996) Microorganisms in honey. Int. J. Food Microbiol. 31: 1-26.

Tasneem P. and K. Aruna (2013). Microbiological analysis, biochemical composition and antibacterial activity of crude honey against multiple drug resistant uropathogens. Research Journal of Pharmaceutical, Biological and Chemical Sciences; 4(3):434-444 (AN: 20133312132).

Wajiha Gul.; N. Farooq; U. Khan; F. Rehan and D. Anees (2015). Honey: A nectarous anti-infective agent. World J. of Pharmacy and pharmaceutical Sciences; 4 (4): 208-215 (ISSN 2278 – 4357).

Whadan, H. A. (1998). Causes of the antimicrobial activity of honey. Infection; 26: 26-31.

ARABIC SUMMARY

العلاقة بين فترات التخزين والكائنات الدقيقة (البكتيريا والفطريات والخمائر) في العسل

محمود عبد السميع محمد علي[١]- روداء رمضان خليل[١]- هيثم محمودرمضان [٢]
١- قسم وقاية النبات- كلية الزراعة - جامعة عين شمس – القاهرة – مصر
٢- قسم وقاية النبات - كلية الزراعة - جامعة طنطا – الغربية – مصر

تم تقييم العلاقة بين فترات التخزين والكائنات الدقيقة (البكتيريا والفطريات والخمائر) في عينات العسل. تم جمع العسل الناضج من خلايا نحل العسل التي تتغذى على الزهور الطبيعية في محيط منطقة الدراسة بالإضافة إلى محلول سكري (١: ١). كان عدد البكتيريا ١,٤٠ ، ١,٨٠ ، ٢,٥٣ ، ٣,٤٧ و ٤,٩٠ مستعمرة/عينة، وكانت ٠,٣٠ ، ٠,٥٠ ، ٠,٩٠ ١,٦٠ و ٢,٢٠ مستعمرة/عينة للفطريات و ٢,٧٠ و ٣,١٠ و ٣,٩٠ و ٤,٥٠ و ٧,٢٠ مستعمرة/عينة للخمائر في عينات العسل المخزنة عند صفر و ٣ و ٦ و ٩ و ١٢ شهراً، على التوالي. وفقاً لإجراءات العزل والتعريف للكائنات الحية الدقيقة في عينات العسل المختبرة، ثلاثة أنواع من البكتيريا *Bacillus brevis*، *Bacillus cereus* و *Clostridium botulism*، أربعة أنواع من الفطريات *Aspergillus apis*، *Aspergillus niger*، *Cladosporium* sp. و *Penicillium* sp. وثلاثة أنواع من الخمائر *Debaromyces* sp.، *Lipomyces* sp. و *Saccharomyces* sp. تم تعريفها وفقا لصفات وخصائص وفسيولوجيا المزرعة. كما لخصت النتائج أن الفطريات كانت أقل تعدادبالمقارنة بالبكتيريا والخمائر. وكانت بكتريا *Clostridium botulism*الأكثر تكراراً (٪) مقارنة مع أنواع البكتيريا الأخرى. كما كان فطر *A. apis* الأكثر تكراراً مقارنة مع أنواع الفطريات الأخرى، كما كانت الخميرة.*Lipomyces*sp الأكثر تكرارا، في حين أن الخميرة *Saccharomyces* sp. كانت أقل تكراراً. لخصت النتائج أيضاً أن تعداد وتكرار (٪)الكائنات الحية الدقيقة ازدادتبزيادة فترة التخزين.

الكلمات الدالة: العسل، التخزين، الكائنات الدقيقة، البكتريا، الفطريات، الخمائر