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# Evaluation of the Sewi dates safety produced by the traditional method

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#### Abstract

Sewi dates which is traditionally produced by sun drying at open air are exposed to contamination by various contaminants such as soil particles, dust, pests, birds and other contaminants like heavy metals, aflatoxins and microorganisms. The present work aimed to evaluate the level of heavy metals contamination, and microbial load as well as aflatoxins in Sewi dates samples produced by traditional methods. Thirty samples of Sewi date were collected from different local markets representing ten sites at Giza governorate. The date samples were digested by microwave oven (closed system), then the levels of heavy metals were determined by ICP. Also, microbiological analyses (total bacteria counts and yeasts and molds count) of Sewi date samples were assessed. Also, Levels of aflatoxins were determined by HPLC. The contamination levels of date sample by heavy metals, aflatoxins and microbial counts varied according to sampling location. The results showed that most of the collected date samples had high contamination with heavy metals, aflatoxins and microbes. Some metals such as Pb, Cd and Ni were above the maximum residue limits (MRLs), while As and Hg levels were below the detection limits in all date samples. Total bacterial count in Sewi date ranged from 2.537 to 4.85 Log CFU/g. while, mold and yeasts recorded lower counts that ranged from 2.61 to 3.973 Log CFU/g. In contrast, no coliforms, *E. coli* and *Salmonella* were detected in all date samples. The highest detected aflatoxins were AFB<sub>1</sub> with 1.09  $\mu$ g kg<sup>-1</sup>, while no AFB<sub>2</sub> and AFG<sub>2</sub> were detected. Finally, it can be concluded that the modification of date's production is necessary to reduce the contamination levels in the Sewi date to obtain the safe food.

Keywords: Sewi dates - microbial load - heavy metals - aflatoxins - contaminants.

# Introduction

Dates of the date palm (Phoenix dactylifera L.) have always played an important role in the economic and social lives of people especially at south of Giza governorate. Dates are very rich in nutritive components, such as carbohydrates, fats, minerals, proteins, vitamins and dietary fibers. It is preferable to consume dates at the Rutab (semi-ripe) and Tamr (fully-ripe) stages [1]. One of the basic reasons for monitoring the levels of contaminants in dates was the dramatic increase in environmental pollution in the recent years. Regarding the contamination of dates with heavy metals, the rapid increasing population in urban areas led to anthropogenic activities and fossil fuel combustion. Also, emissions from road traffic that uses fossil fuel, industry, agriculture sewage sludge, and waste incineration are the chief sources of air pollution [2, 3].

Microbial contamination of date with yeasts, molds, and bacteria is a major obstacle facing local and international marketing of Egyptian dates produced by traditional methods [4]. Dates are fairly dry fruits, with water and sugar contents of 10-15% and 60-88% (on dry basis), respectively [5], hence they are generally regarded as stable to microbial spoilage. However some contaminants, especially osmotolerant yeasts and molds, may survive for longer times or even grow on the fruits. Potential microbial contaminants isolated from date fruits include yeasts, molds, and some pathogens bacteria such as *Staphylococcus aureus*, *E. coli*, *A. flavus* and *A. parasiticus* [6].

Date fruits are prone to fungal contamination in the field; during harvest, transport, and marketing; and with the consumer. Fungi play a substantial role in spoilage of date fruits, because of their pathogenicity to the harvested products. Fungi (*Aspergillus, Alternaria*, and *Penicillium* spp.) may grow on high-moisture dates, especially when harvested following rain or high humidity periods [7, 8]. Mycological profiles for date fruits were studied previously by several studies such as Ragab et al. [9], Iamanaka et al. [10], Al-Sheikh [11], Bokhary [12]

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and Gherbawy et al. [13]. Mycotoxins are a group of toxic fungal secondary metabolites which can contaminate agricultural products under pre- and postharvest conditions. Aflatoxins  $B_1$ ,  $B_2$ ,  $G_1$  and  $G_2$  exist predominantly in dried fruits such as date [14, 15].

Generally, most of the Egyptian Sewi dates (Agwa) as semi dry dates are produced by removing the original water content naturally, through sun drying for 10-15 days at open system (the traditional method). The produced dates at open system exposed to dust, soil particles, pests, birds and animals. So, the produced Sewi dates can be contaminated by many of contaminants such as heavy metals, aflatoxins, yeasts, molds and bacteria. Therefore, production of Agwa dates lacks to food safety systems, which is negatively affect both the consumers' health and the export rate. This work aims to estimation the levels of the different contaminants such as heavy metals, aflatoxins and microbiological analyses in the Sewi dates produced by the traditional method.

#### Materials and Methods 1. Sampling

Thirty samples of Sewi date were collected from different local markets representing ten sites at Giza governorate (Atfih, Masjed Mossa, Soal, Al-Saff, Faisal, Dokki, Bolak-Aldakror, Al-Harm, Al-Moatamadiah and Ard-Alowaa) as three replicates for each site. The date samples (each sample 1 kg package) were collected and stored at 4°C in a refrigerator until laboratory analysis.

# 2. Heavy metals analysis

# 2.1. Sample preparation

The microwave (closed system) was used for digestion of date samples according to European Committee for standardization [16]. Briefly, a 0.5 g of homogenized date sample was weighted and transferred in the PTFE vessels for microwave digestion. Subsequently, 8 ml of nitric acid (69%) and 2 ml  $H_2O_2$  were added to the sample. The vessel was closed completely and excellently, and then transferred to the microwave until complete of digestion. Digestion was performed in the microwave oven by temperature-controlled program: heating to 200°C for 15 min, holding time 15 min, cooling to 85°C for 15 min. After cooling to room temperature, the content of the vessel was transferred to a volumetric flask (25 mL) and diluted with ultrapure water to the mark, then ready for analysis by Inductive Coupled Plasma Optical Emission Spectrometry (ICP-OES).

# 2.2. Determination of heavy metals

Analysis for investigated heavy metals was performed at Water Pollution Department, National Research Centre using the Agilent 5100 Synchronous Vertical Dual View (SVDV) ICP-OES according to APHA [17] with Agilent Vapor Generation Accessory VGA 77. For each series of measurements, the intensity calibration curve was constructed composed of a blank and three or more standards from Merck Company (Germany). Accuracy and precision of the Fe, Mn, Cd, Pb, Ni, Cr, As, Hg, Zn and Cu ions measurements were confirmed using external reference standards from Merck, and standard reference material and quality control sample from National Institute of Standards and Technology (NIST) were used to confirm the instrument reading.

# 3. Microbiological analyses

Representative sample (10 g) was taken and homogenized in 90 ml of 0.9% NaCl for 30 s. Serial 10 fold dilutions were prepared in saline tubes, and 1 ml of solution was used for microbial count. Microbial enumeration was performed using pour plate method according to APHA [18] and FDA [19].

# 3.1. Total viable count

Total viable count was performed using the plate count agar medium as recommended by APHA [18] and FDA [19]. Dilutions were made which were transferred to sterilized plates (1 ml per plate approximately). At the end of 2 days of aerobic incubation at  $35^{\circ}$  C, the plates were analyzed for total growing population (CFU/gram).

# 3.2. Coliforms count and *Escherichia coli* examination

Coliforms count and *Escherichia coli* examination were performed as described by El-Hadedy and Abu El-Nour [20]. For each sample, 9 ml Bromo-Cresol Purple MaCconkey broth tube was inoculated with 1 ml of the first three dilutions (3 tubes for each dilution). Positive tubes were indicated by the production of acid and gas upon incubation at 37°C for 24 hours, and the count was estimated statistically from the MPN index [21]. From each positive tube, a new MaCconkey broth tube was inoculated with one loopful, and incubated at 45.5°C for 24 hours, to detect the fecal coliforms by the presence of acid and gas. EMB Agar plate was streaked from the positive tube (45.5°C), and *Escherichia coli* was distinguished as purple colonies with green metallic sheen [22].

Egypt. J. Chem. 65, No. 12 (2022)

# 3.3. Detection of Salmonella

Detection of Salmonella was performed according to APHA [18] and FDA [19]. As 25 g of properly homogenized sample were added to 225 ml of sterile buffered peptone water for pre-enrichment. After incubation at  $37^{\circ}$  C/24 h, 10 ml of growth suspension were transferred to 90 ml boiling sterilized Selenite broth supplemented with 4 g l<sup>-1</sup> sodium bi-selenite (Oxoid) and incubated at  $37^{\circ}$ C/24 h. After incubation, XLD plates were streaked from selenite broth [23, 24]. Colonies were detected as *Salmonella* when appeared red with black centers.

#### 3.4. Molds and yeasts counts

Molds and yeasts counts were examined using malt extract agar [25]. Dilutions were made which were transferred to sterilized plates (1 ml per plate approximately). At the end of 3 days incubation period at  $30^{\circ}$ C, the plates were analyzed for yeast and fungal population (CFU/gram). Growing colonies morphology on specific agar media, and microscopic examination for target colonies were performed [18, 19].

#### 4. Aflatoxins determination

Aflatoxins AFB<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> standards were used throughout the present study (Sigma, chemical company, USA). Extraction, clean up and determination of aflatoxins was done according to AOAC [26]. Aflatoxins (AFs) were extracted from date samples according to CB method [26] as follows: 50 g of each sample was put into 500 ml Erlenmeyer flask containing 25 g diatomaceous earth, 250 ml chloroform and 25 ml distilled water. The mixture was shaked with a horizontal shaker for 30 min. The extract was filtered through filter paper Whatman No 4. The first 50 ml of extract was collected and transferred for clean up using silica gel column. Chromatographic columns were prepared by initially packing anhydrous sodium sulphate (5 g) into a glass column (22 x 300 mm) with plug of glass wool. Chloroform was added to 10 g of silica gel for column to create slurry, which was added to the chromatographic column. The stopcock was opened to allow the silica gel packing to settle, while the excess chloroform was drained. During draining, another 10 g anhydrous sodium sulphate was added to the top of silica gel to prevent column from drying. A portion of filtrate, 50 ml, was loaded to the column and allowed to flow at a rate of a drop/second. The extract was rinsed with 150 ml n-hexane followed by 150 ml diethyl ether. Aflatoxins were eluted with 150 ml chloroform: methanol (97:3 v/v) into 250

Erlenmeyer flask and then was evaporated to dryness using rotary evaporator. The residue was quantitatively transferred to a small vial with chloroform and evaporated to dryness under nitrogen.

Two hundred  $\mu$ l quantities of hexane were added to the cleanup dry film of samples followed by 50  $\mu$ l trifluoroacetic acid (TFA) and they are mixed well for 30s. The mixture was let to stand for 5 min, then the mixture, 450 ml H<sub>2</sub>O: CH<sub>3</sub>CN (9:1 v/v) were added and mixed well by vortex for 30s. The mixture was left to stand for 10 min to form two separate layers. The lower aqueous layer was used for HPLC analysis.

The HPLC system used for Aflatoxins determination was Agilent, series 1260 system with (Germany), equipped quaternary pump, fluorescence detector and а C18 column chromatography Phenomenex (250 x 4.6 mm, 5 µm). The mobile phase was water: methanol: acetonitrile (60:30:10) using as isocratic flow rate of 1.2 ml min<sup>1</sup> at 360 nm excitation and 440 nm emission.

# 5. Statistical analysis

Results were subjected to one-way analysis of variance (ANOVA) of the general linear model (GLM) using SAS [27] statistical package. The results were the average of three experiments ( $p\leq 0.05$ ).

## **Results and discussion**

## 1. Levels of heavy metals in the Sewi date samples

The main sources for date contamination by heavy metals are contaminated soil, polluted air, pesticides, fertilizers and irrigation by wastewater. Heavy metals can be taken up by date through adsorption from a contaminated soil or by surface deposition from a polluted air [28, 29].

## 1.1. Levels of toxic metals in the Sewi date samples

Levels of As and Hg were below the detection limits (0.001 mg kg<sup>-1</sup>) in all date samples. Regarding the levels of Pb in the Sewi date collected from Giza governorate, the results in Table (1) reveal that the samples of Masjed Mossa zone recorded the highest level of Pb with mean concentration 2.045 mg kg<sup>-1</sup>. Meanwhile, date samples of Al-Moatamadiah zone recorded the lowest level of Pb with mean concentration 0.353 mg kg<sup>-1</sup>. In general, all detected levels of Pb in the date samples were above the maximum residue limit (MRLs) as 0.1 mg kg<sup>-1</sup> according to European Commission [30]. The concentration of Pb in the date fruits depends on the relative level of exposure from a contaminated soil in addition to the phyto-availability and deposition of the Pb from the polluted air [31].

The mean levels of Cd in the Sewi date samples ranged from < d.l. (0.001 mg kg<sup>-1</sup>) for Al-Moatamadiah and Ard-Alowaa samples to 0.262 mg kg<sup>-1</sup> for Masjed Mossa samples (Table 1). The detected levels of Cd in the date samples collected from Atfih, Masjed Mossa, Soal, Al-Saff, Bolak-Aldakror and Al-Harm zones were above MRLs as 0.5 mg kg<sup>-1</sup> according to European Commission [32]. On the other hand, the detected levels of Cd in the date samples collected from Dokki, Faisal, Al-Moatamadiah and Ard-Alowaa zones were below MRLs. The main sources of contamination with Cd phosphate fertilizers, are industrial wastes, agrochemical wastes, pigments, tobacco and rechargeable batteries [33, 34].

Unlike Pb and Cd, the mean concentrations of Cr in all collected Sewi date samples were below MRLs of WHO/FAO [35] as 1.3 mg kg<sup>-1</sup>. The highest mean level of Cr in Sewi date was recorded in Al-Harm samples as 1.07 mg kg<sup>-1</sup> with significant difference

between them and the other zones. Meanwhile, the date samples of Soal recoded the lowest mean value as 0.503 mg kg<sup>-1</sup>. In contrast, the mean concentrations of Ni in most of the collected Sewi date samples (70%) were above MRLs of WHO [36] as 1.5 mg kg<sup>-1</sup>. Generally, the detected levels of Ni in the collected Sewi date ranged from 0.93 to 3.04 mg kg<sup>-1</sup>. The main source of Ni is the irrigation water as reported by Abdel-Razek [37] who noticed that the level of Ni in the irrigation water was 73.2 mg  $\Gamma^1$ . Also, Ni can be accumulated from agricultural soil to different parts of plant [38].

The variation of toxic metals levels between Sewi date samples may be return to environmental condition of the different zones. For example, fruits growing at the roadside may be accumulating the toxic metals, especially from vehicle emissions as suggested by Feng et al. [39] and Abdel-Rahman et al. [40]. Also, Shahid et al. [41] disclosed that airborne heavy metals may be deposited and absorbed on the different parts of the plants. As well as, Sulaiman and Hamzah [42] who reported that the uptake and translocation of some metals such as Cd, Cu, Fe, and Pb in different parts of roadside plants were higher than those grown in uncontaminated site.

Sample location	Mean $\pm$ S.E. (mg kg <sup>-1</sup> dry weight)					
	Pb	Cd	Cr	Ni		
Atfih	$1.644^{\circ} \pm 0.067$	$0.134^{\circ} \pm 0.012$	$0.614^{d} \pm 0.023$	$2.83^{a} \pm 0.11$		
Masjed Mossa	$2.045^{a} \pm 0.055$	$0.262^{a} \pm 0.021$	$0.772^{\circ} \pm 0.031$	$3.04^{a} \pm 0.10$		
Soal	$1.898^{b} \pm 0.044$	$0.186^{b}\pm 0.014$	$0.503^{e} \pm 0.021$	$2.89^{a} \pm 0.13$		
Al-Saff	$1.671^{a} \pm 0.054$	$0.247^{a} \pm 0.016$	$0.583^{\ d} \pm 0.016$	$2.48^{b} \pm 0.14$		
Faisal	$0.673^{\rm \ f} \pm 0.031$	$0.011^{e}\pm0.007$	$0.316^{g} \pm 0.027$	$1.77 ^{\rm c} \pm 0.08$		
Dokki	$0.745^{\rm \; f}\pm 0.072$	$0.005^{e} \pm 0.002$	$0.269^{g} \pm 0.017$	$1.35^{d} \pm 0.07$		
Bolak-Aldakror	$1.255^{d} \pm 0.039$	$0.092^{d} \pm 0.009$	$0.858^{\ b}\pm 0.021$	$1.29^{d} \pm 0.07$		
Al-Harm	$1.037^{e} \pm 0.054$	$0.067^{d} \pm 0.008$	$1.070^{a} \pm 0.019$	$1.92^{\circ} \pm 0.10$		
Al-Moatamadiah	$0.353^{\ h} \pm 0.022$	< d.l.	$0.384^{\rm f} \pm 0.016$	$0.93^{e} \pm 0.06$		
Ard-Alowaa	$0.523^{\ g} \pm 0.021$	< d.1.	$0.420^{\rm \ f} \pm 0.027$	$2.30^{b} \pm 0.07$		
LSD	0.144	0.033	0.065	0.284		

TABLE 1. Levels of toxic metals in the Sewi date samples collected from Giza governorate.

Means followed by different subscripts within column are significantly different at the 5% level. LSD: Least Significant Difference. < d.l.: below detection limit.

# 1.2. Levels of essential metals in the Sewi date samples

Sewi date is a good source for essential metals such as Fe, Zn, Cu and Mn. For example, Zn is necessary for sustaining all life. It is a trace element in the diet, forming an essential part of many enzymes, and playing an important role in protein synthesis and cell division [43]. With regard to the levels of essential metals in the Sewi date samples, the results in Table (2) revealed that the mean concentrations of Cu in all collected samples were below MRLs of Codex [44] as 10 mg kg<sup>-1</sup>. The highest mean levels of Cu in Sewi date were recorded in Bolak-Aldakror, Soal and Masjed Mossa samples as 5.42, 5.05 and 5.02 mg kg<sup>-1</sup>, respectively with no significant difference between them. In contrast, the date samples of Al-Harm and Al-Moatamadiah recoded the lowest mean values as 1.82 and 2.18 mg kg<sup>-1</sup>, respectively with no significant difference between them.

Zinc occurs naturally in water and soil, but Zn concentrations are increasing unnaturally as a result of human activities. Levels of Zn in the collected Sewi date samples ranged from 9.63 mg kg<sup>-1</sup> at Faisal zone to 31.40 mg kg<sup>-1</sup> at Atfih zone (Table 2). These results are in agreement with Tamirat

[45], who reported that the levels of Zn ranged from 9.27 mg kg<sup>-1</sup> to 27.9 mg kg<sup>-1</sup>. Also, Mn helps in formation and activation enzymes. It functions as an antioxidant, helps to grow bones and heals wounds by increasing the development of collagen [45]. Levels of Mn in Sewi date were below the levels of Zn, where the mean concentrations varied from 5.34 mg kg<sup>-1</sup> in Ard-Alowaa samples to 12.96 in Bolak-Aldakror samples. These results are in parallel with Mohamed et al. [46] who noticed that the level of Mn in date ranged between 5.4 and 7.8 mg kg<sup>-1</sup>.

Iron (Fe) plays an important role in plants for chlorophyll growth and in animals; it is a component of hemoglobin that carries oxygen from the lungs into the body's tissues [45]. The Sewi date contains the high levels of Fe as compare with the other determined metals. The date samples of Dokki zone recoded the lowest mean level of Fe as 50.2 mg kg<sup>-1</sup>. Meanwhile, the date samples of Al-Harm zone recoded the highest mean level of Fe as 148.3 mg kg<sup>-1</sup>. These results are similar with Kamal and Fahad [47] who noticed that the level of Fe in date ranged from 80 to 202 mg kg<sup>-1</sup>. The wide variation of essential metals may be return to variation of metals levels in agricultural soil and irrigation water of the different studied zones [48, 49].

Sample location _	Mean $\pm$ S.E. (mg kg <sup>-1</sup> dry weight)				
	Cu	Zn	Mn	Fe	
Atfih	$4.40^{b} \pm 0.13$	$31.40^{a} \pm 0.53$	$7.75^{d} \pm 0.32$	$100.4^{e} \pm 1.1$	
Masjed Mossa	$5.02^{a} \pm 0.17$	$28.31^{b} \pm 0.60$	$9.70^{\circ} \pm 0.32$	$112.8^{\circ} \pm 1.3$	
Soal	$5.05^{a} \pm 0.16$	$30.24^{a} \pm 0.35$	$9.37^{c} \pm 0.26$	$106.2^{d} \pm 1.6$	
Al-Saff	$3.53^{\circ} \pm 0.17$	$26.67^{\circ} \pm 0.31$	$8.07^{d}\pm0.25$	$98.4^{e} \pm 1.1$	
Faisal	$2.86^{d} \pm 0.12$	$9.63^{\ f} \pm 0.48$	$11.50^{b} \pm 0.21$	$123.8^{b} \pm 2.1$	
Dokki	$2.71^{d} \pm 0.13$	$25.69^{\circ} \pm 0.56$	$6.52^{e} \pm 0.22$	$50.2^{\text{g}} \pm 1.9$	
Bolak-Aldakror	$5.42^{a} \pm 0.17$	$14.38^{e} \pm 0.62$	$12.96^{a} \pm 0.25$	$76.8^{f} \pm 1.2$	
Al-Harm	$1.82^{e} \pm 0.17$	$16.48^{d} \pm 0.64$	$6.29^{e} \pm 0.19$	$148.3^{a} \pm 1.6$	
Al-Moatamadiah	$2.18^{e} \pm 0.14$	$14.52^{e} \pm 0.33$	$9.77^{c} \pm 0.21$	$145.7^{a} \pm 1.7$	
Ard-Alowaa	$2.85^{d} \pm 0.14$	$14.61^{e} \pm 0.53$	$5.34^{\rm f} \pm 0.24$	$76.8^{f} \pm 1.4$	
LSD	0.445	1.50	0.739	4.53	

TABLE 2. Levels of essential metals in the Sewi date samples collected from Giza governorate.

Means followed by different subscripts within column are significantly different at the 5% level. LSD: Least Significant Difference.

# 2. Microbiological evaluation of the Sewi date samples.

Bacteria, yeast and molds are considered to be the major causative agents of the spoilage of date fruits at all stages of ripening on trees, as well as during storage and processing [50]. The type and level of microorganisms found in food reflect its quality as well as its shelf life. The data obtained from microbiological analyses of Sewi date samples collected from different locations in Giza province was illustrated in Table (3). The total bacterial count ranged from 2.54 to 4.85 Log CFU/g at Atfih and Al-harm locations, respectively with significant differences between them. However, Bolak-Aldakror recorded 4.82 Log CFU/g for total bacterial count with no significant difference with that recorded in Al-harm samples. About 60% of tested site recorded total bacterial counts below 4.0 Log CFU/g, while the other 40% of tested sites recorded over 4.0 Log CFU/g but not exceed 5.0 Log CFU/g. it is worth to mention that no

coliforms, *E. coli* and *Salmonella* were detected in all tested samples which indicate the samples are free from pathogenic bacteria.

The yeasts and molds count ranged between 2.61 and 3.97 Log CFU/g for Atfih and Bolak-Aldakror locations, respectively with significant differences between them. It was observed Atfih and Masjed Mossa locations recorded the lowest count for total bacteria as well as yeasts and molds count as it considered as production sites. Hasnaoui et al. [51] surveyed the microbial quality of dates grown in Morocco and concluded that the total microbial count was low in the range of 102 CFU/g except in some samples. Also, Bacillus cereus, E. coli and coliforms were not detected in any sample. While yeasts and molds were detected in all samples which in agreement with our findings. Molds considered the major causative spoilage agent of dates starting from the ripening stage on the tree as well as during the processing and storage [52].

 TABLE 3. Microbiological analyses of Sewi date samples collected from different locations in Giza governorate.

	Microbial counts Log CFU/g (Mean ± SE)				
Sample location	Total bacterial count	Mold and yeasts	Total coliforms count	E. coli	Salmonella
Atfih	$2.54^{i} \pm 0.029$	$2.61^{\ h}\pm 0.015$	Nil	Negative	Negative
Masjed Mossa	$3.31^{h} \pm 0.018$	$3.48^{\ fg} \pm 0.026$	Nil	Negative	Negative
Soal	$4.18^{\circ} \pm 0.006$	$3.85^{b} \pm 0.012$	Nil	Negative	Negative
Al-Saff	$3.62^{g} \pm 0.017$	$3.50^{ef} \pm 0.021$	Nil	Negative	Negative
Faisal	$3.95^{d} \pm 0.009$	$3.54^{e} \pm 0.003$	Nil	Negative	Negative
Dokki	$4.48^{\ b}\pm 0.015$	$3.58^{d} \pm 0.009$	Nil	Negative	Negative
Bolak-Aldakror	$4.82^{a} \pm 0.006$	$3.97^{a} \pm 0.009$	Nil	Negative	Negative
Al-Harm	$4.85^{a} \pm 0.012$	$3.53^{e} \pm 0.023$	Nil	Negative	Negative
Al-Moatamadiah	$3.85^{e} \pm 0.009$	$3.44^{g} \pm 0.006$	Nil	Negative	Negative
Ard-Alowaa	$3.78^{\rm f} \pm 0.015$	$3.74^{\circ} \pm 0.012$	Nil	Negative	Negative
LSD	0.044	0.046			

Means followed by different subscripts within column are significantly different at the 5% level. LSD: Least Significant Difference.

Also, Hasnae et al. [53] study the microbial quality of dates in the North center region of Morocco and found that the average value for total aerobic bacteria ranged from 0.6 to 4.9 Log CFU/g and the same range was observed for yeasts, while the molds count ranged from 0.5 to 3.8 Log CFU/g. Additionally; no fecal coliforms, total coliforms, Staphylococcus aureus and Salmonella spp. were detected in all tested samples with accordance of our findings. Al Jasser [54] found that aerobic bacterial count of four date varieties (Tamr stage) was ranged from 2.55 to 3.69 Log CFU/g and total molds and yeasts count was ranged between 2.39 and 3.41 Log CFU/g. Also, Ragab et al. [9] reported from 310 - 3030 CFU/g as a range of total molds count in semi dry Saidy date in Egypt. The presence of bacteria, yeasts and molds could be due to nutritional (sugar) content of the Sewi date fruits which may serve as good source of carbon as well as poor hygienic practices during processing. These observations agreed with those reported by Hamad [6] and Omogbal et al. [55].

## 3. Aflatoxins in the Sewi date samples.

The level of Aflatoxins contamination in dried Sewi date samples are illustrated in Table (4). The results showed that out of 10 sites of date samples, 5 sites (50%) were contaminated with  $AFB_1$  and only one site (10%) contaminated with  $AFG_1$  at level 0.16  $\mu$ g kg<sup>-1</sup>. The highest concentration of AFB<sub>1</sub> (1.09  $\mu$ g kg<sup>-1</sup>) was recorded by Bolak-Aldakror, followed by Al-Moatamadiah and Al-Saff with AFB<sub>1</sub> level 0.88 and 0.71  $\mu$ g kg<sup>-1</sup>, respectively. Meanwhile, the lowest AFB<sub>1</sub> level (0.32 and 0.48  $\mu$ g kg<sup>-1</sup>) was found in samples collected from Atfih and Al-Harm, respectively. No aflatoxins were detected in Masjed Mossa, Soal, Faisal, Dokki and Ard-Alowaa locations.

Ragab et al. [9] reported the presence of aflatoxins in 40 samples of different types of raw, treated and processed date collected from Upper Egypt and found that 2 from 5 samples of pitted date stuffed with peanut were contaminated by AFB<sub>1</sub> at a level of 4.8 and 6.2  $\mu$ g kg<sup>-1</sup>. Also, Abdallah et al. [56] found that only one sample from 28 samples of dried date palm fruit collected from different markets in Assiut, Egypt was contaminated with AFB<sub>1</sub> (14.4  $\mu$ g kg<sup>-1</sup>) and AFB<sub>2</sub>  $(2.44 \ \mu g \ kg^{-1})$ . Date samples collected from Tunisian markets during the period from 2012 to 2013, the authors documented 8 types of mycotoxins and found AFB<sub>2</sub> with maximum level of 1.3 and 3.3  $\mu$ g kg<sup>-1</sup>, but no AFB<sub>1</sub> was detected [57]. Iqbal et al. [58] collected 96 date palm products sample from Pakistan and found that AFB<sub>1</sub> in 38 samples (39.6%), where up to 26.6  $\mu$ g kg<sup>-1</sup> and up to 16.7  $\mu$ g kg<sup>-1</sup> in 18 samples (31.6%).

Sample location -	Aflatoxins concentration ( $\mu g k g^{-1}$ )					
	AFB <sub>1</sub>	AFB <sub>2</sub>	AFG <sub>1</sub>	AFG <sub>2</sub>	Total AFs	
Atfih	0.32	< d.l.	0.16	< d.l.	0.48	
Masjed Mossa	< d.l.	< d.l.	< d.l.	< d.l.	< d.1.	
Soal	< d.l.	< d.l.	< d.l.	< d.l.	< d.1.	
Al-Saff	0.71	< d.l.	< d.l.	< d.l.	0.71	
Faisal	< d.l.	< d.l.	< d.l.	< d.l.	< d.1.	
Dokki	< d.1.	< d.1.	< d.l.	< d.l.	< d.1.	
Bolak-Aldakror	1.09	< d.l.	< d.l.	< d.l.	1.09	
Al-Harm	0.48	< d.1.	< d.l.	< d.l.	0.48	
Al-Moatamadiah	0.88	< d.l.	< d.l.	< d.l.	0.88	
Ard-Alowaa	< d.l.	< d.1.	< d.l.	< d.l.	< d.l.	

TABLE 4. Aflatoxins concentration in the Sewi date samples collected from Giza governorate.

< d.l.: below detection limit.

Also, Masood et al. [59] recorded the presence of aflatoxins in 9 out of 15 dried date samples collected from the same area in Pakistan with maximum level 9.8  $\mu$ g kg<sup>-1</sup> for AFB<sub>1</sub> and 18.7 for total aflatoxins. Furthermore, lower level of AFB<sub>1</sub> in date samples was recorded in Iran with level ranging between 0.6 and 6  $\mu$ g kg<sup>-1</sup> in 9 out of 22 date samples [60]. In contrast, a higher level of AFB<sub>1</sub> was observed in 10% of date sample (2 samples) from Yemen within range of 110 to 180  $\mu$ g kg<sup>-1</sup> [61].

Generally, the microbiological, aflatoxins and metal analysis of Sewi date fruits collected from different zones at Giza governorate indicated high contamination of date samples. This may be as a result of exposure to environmental contaminants. Contamination may also occur during increase the drying period (10-15 days) in open system or open sun drying (Fig 1). Thus, the Sewi date fruits produce in Egypt need more hygienic processing such as application of solar energy for drying of date fruits to improve the quality and safety of Sewi dates for human consumption.



Fig. 1. Open sun drying for Sewi dates (OSD).

# Conclusion

From the obtained data, it can be concluded that Pb was detected in all tested samples above the maximum residue limits, however As and Hg were below the detection limit. Heavy metals represent a serious chemical hazard that threatens the safety of produced Sewi dates. No pathogenic bacteria were detected in all samples but the presence of molds can lead to the production of aflatoxins especially AFB<sub>1</sub>. It is worth to mention that the implementation of Good Hygienic Practices (GHP) and Good Manufacturing Practices (GMP), as well as other food safety systems such as application of solar energy for drying of date fruits is essential for the production of safe Sewi dates with high quality.

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Egypt. J. Chem. 65, No. 12 (2022)

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