MONITORING OF PESTICIDE RESIDUES IN DATES PRODUCED FROM DIFFERENT AREAS OF EGYPT

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ABSTRACT

A total of 257 samples of Dates collected from three main cultivated areas namely: "Aswan Governorate (Exclusive famous for dry variety "Bartmoda"), Bahariya Oasis (semi dry variety" Sewi"), and El-Beheira (wet variety" Wet Zaghlul")" were extracted and analyzed for pesticide residues. This was carried out using the accredited (QuEChERs) method as a simple limited monitoring program for use of pesticide residues data in assessment of the possible risks to human health. QuEChERs method allowed the determination of 450 compounds of different pesticide groups. LC-MS/MS and GC-MS/MS were used for residues quantification. Overall, results showed that ca. 46 % of total samples were free from pesticide residues, while 54.09% were contaminated with pesticides, of which 25.29% contained pesticide residues that exceeded maximum residue limits (MRL). The study also analyzed the percentage of total contamination in each area which represented 49%, 30% and 21% in "Bahariya Oasis, Aswan and El- Beheira" respectively. The obtained data indicate that there was no risk of exposure observed in dates samples.

Keywords: Dates, Mass spectrometers, liquid chromatography, Gas chromatography, pesticide residues, maximum residue limit (MRL), QuEChERS, Risk of exposure.

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INTRODUCTION

Palm dates have been known since old ages as a tree with many unique qualities, and man has seen it as a source of good and blessing, in view of the important role that Palm dates have played in the lives of peoples and nations. Palm dates in the Nile Valley have been known since prehistoric ages (Egyptian Agricultural Bulletin No. 1292 of 2013). Dates have full nutritive value and considered to be a principal food in Arab countries, as it is mentioned in the Holy Qur'an. Date palm fruits are very rich in nutritive components, carbohydrates, fats, minerals, proteins, vitamins and dietary fibers, as well as used in folk medicine for treatment of liver diseases and are highly recommended to be consumed by pregnant women before and after delivery (Vayalil, 2012).

Many diseases and pests can infect palm trees, which vary in severity and intensity at different stages of growth. Therefore, pesticides play an important role in preventing crop losses and controlling vectors of diseases. Pesticide residues are present in the majority of fruits and vegetables, which ultimately reach the consumer and slowly cause health hazards, ranging from short-term impacts such as headaches and nausea, to chronic impacts, such as various cancers, birth defects, infertility, and endocrine disruption (Mood *et al.*, 2012). During many years, the suppression of insects relied on the use of chemical products. However, the use of pesticides has many disadvantages such as emergence of resistance towards many active ingredients and

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especially residues in crops (Sabrine *et al*., 2019). The use of pesticides to control pests and different diseases can lead to the presence of pesticide residues. The level of these residues can be below the maximum residue limit (MRL) if good agricultural practices (GAP) were used. The presence of residues with level exceeding MRLs should be interpreted as violation of GAP (Bayoumi *et al* ., 2006).

Nevertheless, the extensive uses of pesticides have raised serious concern for the side effects inflicted on consumers (Abhilash and Singh, 2009). It is already well established that long exposure to low doses has been linked to human risks such as immunity suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Wiles *et al*., 1998).

The improper use of pesticides leads to environmental and food security risks, and monitoring pesticide residues helps to assess the risks of consumer exposure to such residues and ensure a high level of consumer protection (Abd El-Mageed *et al*., 2021). There should be some sort of a cleaning process before arriving to the supermarkets to ensure we reduce the harm if not completely abolish it (Mfarrej *et al*., 2017).

The present work is portraying the result of pesticide residues monitoring program in dates cultivated in the main production areas of date palms in Egypt, and the use of health index module to reflect possible risks to consumers.

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METHODOLOGY

PESTICIDES REFERENCE STANDARDS: Certified reference standards of pesticides were of > 98% purity and purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

REAGENTS: Solvent and chemicals described in the standard method CEN 275, 2007.

SAMPLING: A total of 257 samples of three Dates varieties collected randomly from three markets of main cultivated areas of date palms in Egypt namely "Aswan Governorate (dry variety" Bartmoda"), Bahariya Oasis (semi dry variety" Sewi"), El-Beheira (wet variety "wet Zaghlul") throughout 2019-2020. Representative samples of dates were prepared for residue analysis; 1 kg of each type of dates was prepared according to guidelines of the Codex Committee on Pesticide (CAC/GL 33-1999).

PESTICIDE RESIDUES ANALYSIS: Pesticide residues analysis was carried out in the Central Laboratory of Residues Analysis of Pesticides and Heavy Metals in Food - Ministry of Agriculture, Egypt. The standard method of European Committee for Standardization/Technical Committee 275 (2007) for foods of plant origin: prEN 15662 (QuEChERS) was followed. The determination of residues carried out using GC-MS/MS and LC-MS/MS after acetonitrile extraction/partitioning and cleanup by dispersive SPE. The method validated 450 compounds using LC-MS/MS and GC-MS/MS.

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Agilent Mass Hunter Workstation software was used for data analysis. All pesticides were detected in the multiple reaction monitoring modes (MRM). Each pesticide has a precursor ion there were two productions determined. One production was used for quantification and the other one was used for qualification The Maximum Reside Limits (MRLs) of Codex Alimentarius were used for comparison when those limits were available. In the absence of Codex MRLs, European limits were used.

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APPARATUS

LC-MS/MS analysis: An Agilent 1200 series liquid chromatograph system equipped with Applied Biosystems (API 4000 Qtrape) tandem mass spectrometers with electrosprary ionisation (ESI) interface was used. Separation was performed on a C18 column ZORBAX Eclipse XDB- C18 4.8mm × 150 mm ,5µm particle size. The injection volume was 25 µl. A gradient elution program was at 0.3 ml/min flow rate, in which one reservoir contained 10 mM ammonium formate solution in MeOH :H2O (1:9 V/V) and the other contained methanol. The ESI source was used in the positive mode, and Nitrogen was used as nebulizer gas , curtain gas ,heater gas and collision gas according to manufacturer's settings ;source temperature was 300°C, ion spray potential 5500V, decluster potential and collision energy were optimized using a Harvard apparatus syringe pump .

GC-MS/MS analysis: Agilent Gas Chromatograph 7980A equipped with tandem mass spectrometer 7000 B Quadrupole, El source was used to perform analysis by using HP-5MS capillary column (30 m length × 0.25 mm id × 0.25 µm film thickness) . Samples were injected in a splitless mode and helium was used as carrier gas (1 ml/min) .injector temperature was 250°C, transfer line temperature was 285 °C , ion source temperature was280°C and quadrupole temperature was 150°C .The GC oven temperature was programmed to initially held at 70°C for 2 min then increased to 150°C at 25°C/min (held for 0 min), and raised to 200°C the rate of 3°C/min (held for

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0 min),then went up from 200 to 280 °C at 8°C/min (held for 10 min)This resulted in a total run time of 25 min and complete separation of all the analytes.

LOD and LOQ: The performance of the analytical method was validated in accordance with EU SANCO guidelines for monitoring pesticide multi-residues to check compliance with existing regulations, especially for European Community.

(SANTE 11312/2021) LOD: Limit of determination (LOD) means the validated lowest residue concentration which can be quantified and reported by routine monitoring with validated control methods; in this respect it can be regarded as the LOQ (see below).

LOQ: Limit of quantitation (LOQ) means the lowest concentration or mass of the analyte that has been validated with acceptable accuracy by applying the complete analytical method and identification criteria. LOQ is preferable to LOD because it avoids possible confusion with "limit of detection". However, in Reg. 396/2005, MRLs that are set at the limit of quantification/determination are referred to as "LOD MRLs". not "LOQ MRLs".

Quality Assurance: Analysis method and instruments were carefully validated as a part of the laboratory quality assurance system and were audited and accredited by the Center of Metrology and Accreditation Finnish Accreditation Service (FINAS) ISO/IEC Guide 17025. The criteria of quality

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assurance were followed to determine the performance of the standard method. The average recoveries tests on different types of pesticides at different concentration levels varied between 70- 120%. The reproducibility expressed as relative standard deviation was less than 25%. The limit of quantification (LOQ) was 0.01 mg/kg, which is equal or below the Maximum Residue Limits (MRLs). The measurement uncertainty expressed as expanded uncertainty and in terms of relative standard deviation (at 95% confidence level) is lower than the default value set by EU (±50%).

RESULTS AND DISCUSSION

Pesticide residues are substances that remain in or on air, water, soil, or food following its use. Even food grown without direct pesticide use can still contain residues due to spray drift from nearby farms, long range air transport, or existing groundwater or soil contamination (Magkos *et al*., 2003).

A total of two hundred fifty-seven of date samples were examined for residues of 450 pesticides. The detected pesticides, minimum, maximum, median of the detected levels, and the numbers and percentages of violated samples collected during 2019 and 2020 are shown in Table (1).

In this present study, a total number of 31 different pesticides were detected in date samples. Overall, results showed that 45.92 % of total samples were free from pesticide residues, while 54.09% of total samples

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were contaminated with pesticides of which 25.29% contained pesticide residues that exceeded maximum residue limits (Table 1).

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Table(1): Pesticide residues detected in Dates samples during 2019 and 2020.

Total samples	Free Samples %	Total Contaminated Samples %	Detected Pesticides	Frequency%	Pesticides Level Median	MRL (mg/Kg)	violated Compound%	violatedsamples %
257	45.91%	54.09%	Acetamipri	5.8%	0.01	0.01	4%	25.29%
			Bentazone	0.4%	0.01	0.03	0%	
			Bifenthrin	0.8%	0.085	0.01	0%	
			Carbendazi	7.4%	0.01	0.1	0%	
			Chlorfenap	0.4%	0.01	0.01	0%	
			Chlorothalo	0.4%	0.03	0.01	0%	
			Chlorproph	6.6%	0.01	0.01	2%	
			Chlorpyrifo	17.5%	0.02	0.05	4%	
			Cyfluthrin	10.1%	0.02	0.02	3%	
			Cypermethr	12.1%	0.01	0.05	2%	
			Difenocona	0.4%	LOQ	0.1	0%	
			Dimethoate	4.3%	0.05	0.01	3%	
			Epoxiconaz	0.4%	0.04	0.05	0%	
			Fenpyroxim	1.2%	0.02	0.01	0%	
			Flusilazole	0.4%	0.01	0.01	0%	
			Imidaclopri	5.8%	0.04	0.05	2%	
			Lambda-Cyhalothrin	5.8%	0.01	0.02 EU	1%	
			Lufenuron	0.8%	0.02	0.01	0%	
			Malaoxon	7%	0.015	0.02	3%	
			Malathion	22.2%	0.02	0.02	8%	
			Metalaxyl	0.8%	0.015	0.05	0%	
			Methomyl	2.3%	0.01	0.01	1%	
			Omethoate	4.3%	0.04	0.01	3%	
			Penconazol	0.4%	0.02	0.01	0%	
			Piperonyl	1.9%	0.01	-----	0%	
			Profenofos	3.1%	0.01	0.01	1%	
			Propiconaz	0.8%	0.005	0.01	0%	
			Pyriproxyfe	0.8%	0.005	0.05	0%	
			Tetramethri	0.8%	0.015	0.01	0%	
			Thiamethox	0.4%	0.05	0.01	0%	
			Thiophanate-methyl	3.9%	0.008	0.1 EU	0%	

LOQ : less than the Limit of Quantification

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Eu: European union MRL 2010
Shadow detected pesticide: Not Registered

Registered pesticide residues detected on crops where they are not permitted may also be a result of contamination from adjacent fields, soil, or water (Centner *et al*., 2014) Deliberate use of nonauthorized pesticides has previously been attributed to low levels of education or awareness and a lack of access to information, limited or incorrect technical support, or failure to correctly read pesticide labels or understand their content (Jardim *et al* ., 2012) Moreover, decisions on the use of preferred pesticides are often also a result of previous experiences related to effectiveness, better costs, and product availability on the farm (Recena *et al*., 2008).

The misuse of pesticides by concerned individuals, in addition to lack of or weak national controlling plans are behind the outbreak of adverse effects in developing countries (Sameeh, 2004). Despite the use of Acaricide in many agricultural crops in Tunisia, this chemical product is not legally registered for the use on palm dates and no official trials were carried out on palm dates to determine its efficacy, PHI or adequate dose. Recent trials in Iranian date palm orchards showed that Fenazaquin sprayed with low dose (0.3%), caused high mortality rate (Sabrine *et al*., 2019). The observed violated date samples imported to United Arab Emirates and contained 3 banned pesticides; it is suggested that banned pesticides might still be frequently used in date for exported countries. These results reflect that a certain proportion of farmers may not follow Good Agricultural Practices (GAP) (Abd El-Mageed, 2017)

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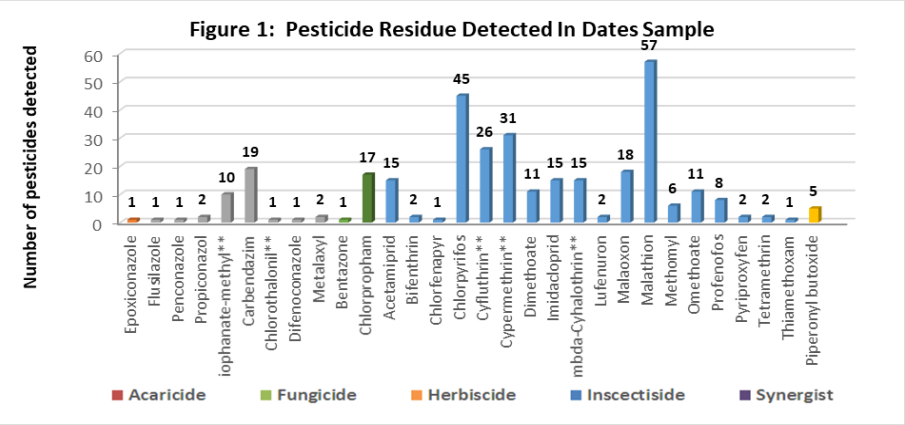
The management of pesticide use and control should be improved. Well-developed training programs should be initiated to improve pesticide application knowledge for farmers. (Yan *et al*., 2018).

Most of the farmers in India use locally made spraying equipment which does not have adequate safeguard and is least durable. This sprayer develops cracks and leaks quite frequently. The use of protective clothing, masks or gloves is also very rare. Further, farmers are ignorant about proper use and efficient application. They are mixing the pesticides that should not be mixed and are using ultra low volume formulations for knapsack sprayers. The equipment of the farmers is not properly maintained, and proper cleansing and correct handling has not been done. Farmers are also not aware of the specific doses of the pesticides to use them in cost effective manner. (Abhilash and Singh, 2009).



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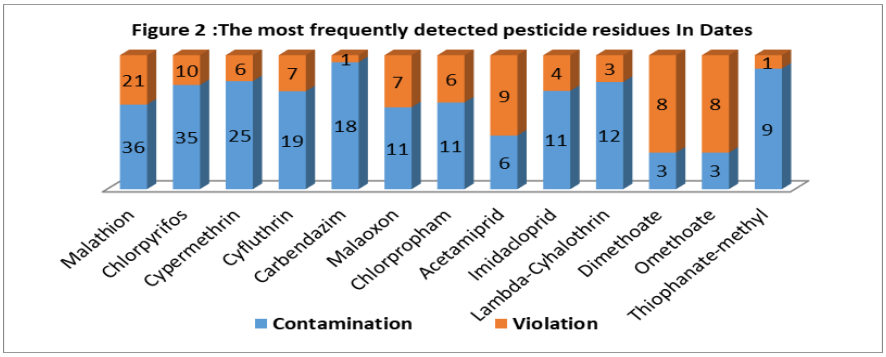
Insecticides, fungicides, acaricides and herbicides have been excessively used in agriculture including date palm farms, which put the health of the consumers at risk with adverse effects (Blanco *et al*., 2002a, b; Colume *et al*., 2000; Fernandez *et al*., 2000, 2001).

Figure (1) showed the highest frequently detected pesticide was Malathion (57 times), followed by chlorpyrifos (45 times), cypermethrin (31 times), cyfluthrin (26 times), carbendazim (19 times), malaoxon (18 times) and Chlorpropham (17 times). Most of pesticide residues detected in Dates were insecticides (58.1%) followed by fungicides (29%), insecticide, herbicide (6.5%), Acaricide (3.2%) and synergist (3.2%).



The most frequently detected pesticide residues were Malathion followed by Chlorpyrifos, Cypermethrin, Cyfluthrin and Carbendazim (figure2).

The following detected Pesticides did not exceed the MRLs (Bentazone – Chlorfenapyr – Difenoconazole – Epoxiconazole – Flusilazole – Metalaxyl – Piperonyl butoxide – Propiconazol – Pyriproxyfen) as shown in the above table.

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Figure 3 showed the most frequently pesticides detected above the MRLs were Malathion, chlorpyrifos and Acetamiprid, Dimethoate, Cyfluthrin, Malaoxon, cypermethrin, Imidacloprid, Lambda-Cyhalothrin, profenofos and Methomyl.

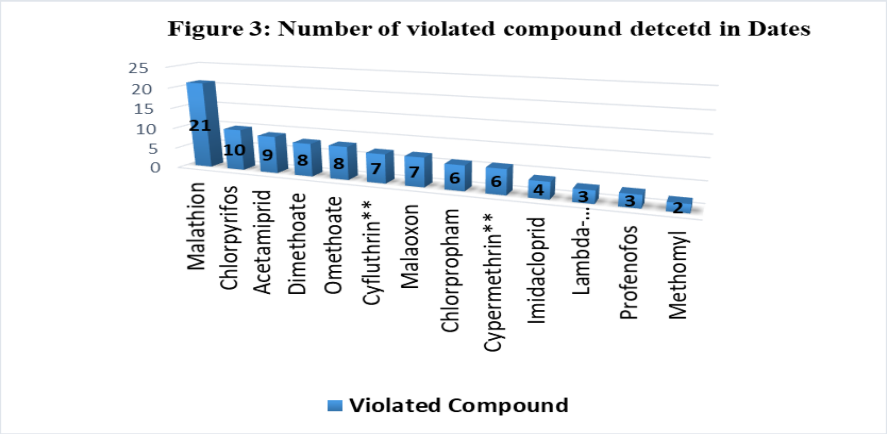

Figure.3 showed the number of violated compounds arranged in descending order as Malathion was the most frequently detected residue in all the samples. It occurred at a mean concentration of 0.02 in 8.17% of date samples followed.

Table(2): Situation of Pesticide Residues in the three main production area of date palms in Egypt

Commodity	Governorates	Total contamination Percentage
Dates	Bahariya Oasis	49%
	Aswan	30%
	El- Beheira	21%

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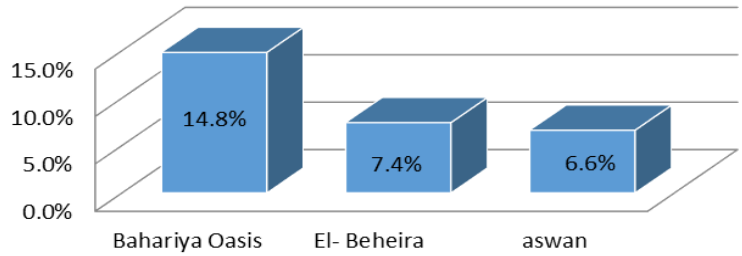
Pesticide residues were detected in 139 samples (54% of total samples) whereas total contamination represented 49%, 30% and 21% in "Bahariya Oasis, Aswan and El- Beheira" respectively. Seventy-Four samples (28.8% of total samples) were found to contain residues below maximum residues limits (MRLs). Data in figure (4) showed the percentage of contaminated samples found in Bahariya Oasis, El- Beheira and Aswan in (14.8%, 7.4% and 6.6%), respectively.

Figure 4 : Percentage of contaminated samples in three main production areas of date Palms



A total of 65 samples (25.29% of total samples) with residues exceeding above MRLs of one or several pesticides were detected as shown in Table 1. Data in figure (5) showed the percentage of violated samples in Bahariya Oasis, El- Beheira and Aswan in (11.7%, 8.9% and 4.7%), respectively.

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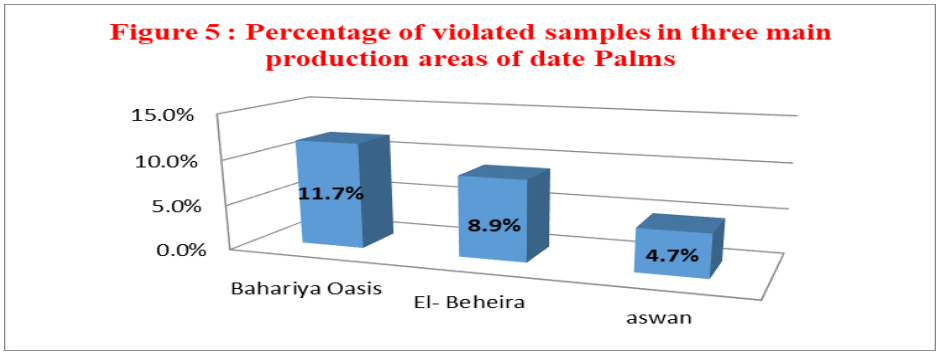
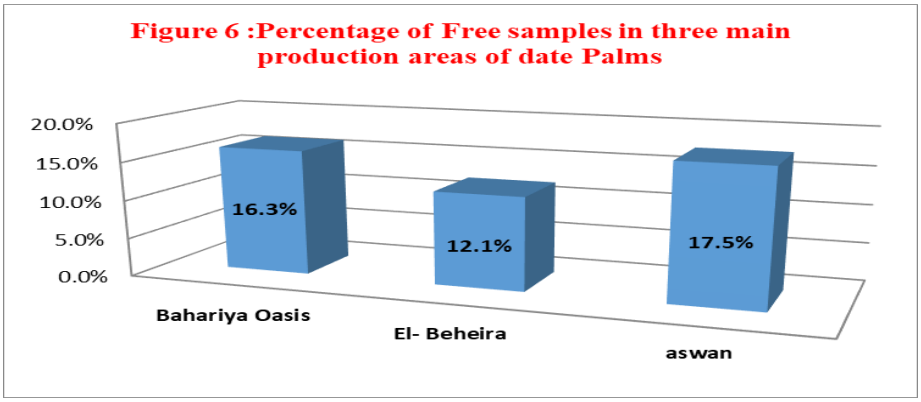


Situation of Free samples in the three main production area of date palms in Egypt: In figure 6, the results show that there have been 118 free samples of pesticide residues in the three main production area of date palms in Aswan, Bahariya Oasis and El- Beheira in (17.5%, 16.3% and 12.1%), respectively.



Risk Assessment and Risk of Exposure: Risk Assessment: Risk assessment is carried out by comparing the concentrations of residues detected, with the established acceptable daily intake (ADI). The level of

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residue concentration in Dates was determined as the arithmetic mean of all the results obtained. Residues higher than the Limit of Quantification (LOQ) were used in the calculation of exposure in order to avoid overestimation of Estimated Daily Intake (EDI). The EDI (mg kg -1 bw-1 day) of each pesticide residue was calculated by multiplying the mean concentration of pesticide residue (mg kg−1), the food consumption rate (kg day−1) and divided by body weight, assuming an average adult's body weight of 60 kg. Meanwhile, the estimated daily intake (EDI) of pesticide residues was calculated as follows:

Pesticide intake (mg/kg bw/day) = [pesticide residue (mg kg-1) ×consumption (kg/day)] ÷Body weight (kg).

Hazard Risk Index (HRI) Analysis: HRI of the residues will be computed using the results and the following equation modified after European Food Safety Authority (EFSA) (European Food Safety Authority, 2013) " HRI = EDI/ADI"

Where EDI is the estimated daily intake, ADI is the acceptable daily intake. HRI value more than 1 is considered as not safe for human health (Darko & Akoto, 2008)**.**

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Table(3): Acceptable daily intake for violated pesticide residues detected in Date samples and the estimated hazard index %

Pesticide	violated conc. (mg/kg)	food consumption (gm/person/day)	EDI	ADI mg/kg bw/day	Hazard index (EDI/ADI, %)	HR
Malathion	0.02	31.5	0.000011	0.03	0.035	NO
Acetamiprid	0.01	31.5	0.000005	0.025	0.021	NO
Cyfluthrin	0.02	31.5	0.000011	0.003	0.350	NO
Malaoxon	0.015	31.5	0.000008	0.03	0.026	NO
Chlorpropham	0.01	31.5	0.000005	0.05	0.011	NO
Cypermethrin	0.01	31.5	0.000005	0.05	0.011	NO
Imidacloprid	0.04	31.5	0.000021	0.06	0.035	NO
Lambda-Cyhalothrin	0.01	31.5	0.000005	0.0025	0.210	NO
Profenofos	0.01	31.5	0.000005	0.03	0.018	NO
Methomyl	0.01	31.5	0.000005	0.0025	0.210	NO
Bifenthrin	0.085	31.5	0.000045	0.015	0.298	NO
Carbendazim	0.01	31.5	0.000005	0.02	0.026	NO
Chlorothalonil	0.03	31.5	0.000016	0.015	0.105	NO
Fenpyroximate	0.02	31.5	0.000011	0.01	0.105	NO
Lufenuron	0.02	31.5	0.000011	0.015	0.070	NO
Penconazole	0.02	31.5	0.000011	0.03	0.035	NO
Thiamethoxam	0.05	31.5	0.000026	0.026	0.101	NO
Thiophanate-methyl	0.008	31.5	0.000004	0.08	0.0001	NO

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-Established by EU Pesticides Database and EFSA (European Food Safety Authority). ADI: Acceptable daily intake; EDI: estimated daily intake; HRI: health risk index, HR: health risk

Few studies on pesticide residues in Dates have been carried out. The obtained results in our study are in agreement with those obtained by El-Saeid and Al-Dosari (2010) for a survey of pesticide residues in date fruits from a market in Riyadh City, Saudi Arabia. They detected fungicide, insecticide and herbicide residue concentrations that violated the MRL values in some samples, whereas chlorpyrifos was found to be the highest residual detected pesticide

Another similar monitoring program was executed in Saudi Arabi whereas 200 date fruit samples collected from different large markets in Al-Qassim region. Pesticide residues were detected in 18% of the date fruits samples, of which 7.5% exceeded the maximum residue levels. The detected pesticide residues do not pose a health risk. (Abdallah *et al*., 2018). Moreover, residue levels of 343 compounds were determined in 230 date samples imported to United Arab Emirates (UAE) during 2020. Results indicated that the percentage of samples with residues above the maximum residue levels (MRL) was 4.34% in dates samples, whereas samples with residues within MRL were 7.39% in dates samples. A total of 230 samples of 88.26% were free from detectable residues. Out of the 343 pesticides tested, 11 pesticides were found above the limit of detection, according to UAE,

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Codex, and European regulations. (Abd El-Mageed *et al*., 2021). In Pakistan, QuEChERS extraction method was optimized and validated for screening of multiclass pesticide residues in date palm fruit samples collected from various markets. Forty percent (40%) of samples were found to be contaminated with various pesticides. The most frequently detected residues were carbofuran, carbaryl, metalaxyl, tebuconazole, triazophos, and pyriproxyfen. The concentration of all the detected pesticides in real samples was below the EU-MRLs. (Qayyum *et al.*, 2021).

Another study has developed a convenient, fast, effective and safe analytical method (QuEChERS) to determine 198 pesticide residues in multi-source date palm fruits using gas chromatography-tandem mass spectrometry (GC-MS/MS). The validated procedure was used to monitor pesticide residues in 30 fresh date samples. The study concluded that the modified QuEChERS extraction method was efficient in analysing pesticide residues in dates palm and none of the samples contained residues above the MRLs. (Khezri *et al*.,2022).

CONCLUSIONS

Pesticide residues monitoring program is a compliance program used to monitor the level of chemical residues of pesticides in three markets of main cultivated areas of date palms in Egypt. The calculated data indicated that there was no risk of exposure observed in date samples.

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From a public health perspective, pesticide residues may pose a potential health risk to consumers. To reduce this risk, it is highly recommended that farmers should be aware by the better use of agricultural pesticide practices, and the need for continuous pesticide residue monitoring.

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تقصى متبقيات المبيدات فى عينات البلح المنزرعة في مناطق مختلفة بجمهورية مصر العربية

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المستخلص

تم تحليل 257 عينة بلح تم تجميعها من ثلاثة مناطق انتاج رئيسية لنخيل البلح في مصر (أسوان – البحيرة – الواحات البحرية) بهدف تقصي متبقيات المبيدات وإجراء تحليل للمخاطر المحتملة التي قد تؤثر على صحة الانسان. تم استخدام طريقة التحليل المعتمدة (QuEChERs) والتي تسمح بتقصي قيم ٤٥٠ مركبًا من المجموعات الكيميائية المختلفة من المبيدات حيث تعتمد طريقة التحليل على استخدام أجهزة التحليل الكروموتوغرافي السائل والغازي عالي القدرة المزود بمطياف الكتلة لتقدير قيم متبقيات المبيدات.

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أوضحت النتائج أن ٤٥,٩٢٪ من إجمالي العينات كانت خالية من متبقيات المبيدات بينما ٥٤,٠٩٪ كانت ملوثة بالمبيدات والتي تضمنت نحو ٢٥,٢٩٪ على متبقيات مبيدات بقيم أعلى من الحدود القصوى المسموح بها.

تم رصد متبقيات المبيدات بقيم تتجاوز الحودد القصوى المسموح بها في عدد ٦٥ عينة من البلح في جميع مناطق الانتاج الرئيسية لنخيل البلح في مصر (أسوان – البحيرة – الواحات البحرية)

تم حساب بيانات التعرض للخطر وفقًا لنتائج تقصي متبقيات المبيدات في الدراسة وكانت جميع العينات الملوثة بما في ذلك التي تجاوزت الحودد القصوى المسموح بها في نطاق ٠,٠٠٠٠١٪ – ٠,٣٥٠٪ مما يؤكد عدم وجود أي تعرض ملحوظ للخطر في عينات البلح .

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