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Pesticide residues surveillance of date palm (*Phoenix dactylifera*) in the south of Tunisia

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Abstract
Phoeniciculture is one of the most important sectors in Tunisia. This palm date is currently in danger due to several constraints such as diseases and Pests. 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 especially residues in crops. The objective of this work is to monitor pesticide residues in dates sampled from Tunisian oasis and to check compliance with existing regulations especially for European Community. The analysis of chromatograms related to seven samples of dates, taken from treated and non treated orchards from the south of Tunisia carried out by extraction procedure based on QuECHERS method followed by UHPLC-MS/MS chromatographic analysis showed that Fenazaquin was the only active ingredient that exceeded the Maximum Residue Levels (MRL) set by the European Union (LD = 0.1 µg.Kg-1). Results showed that the value of concentration of Fenazaquin show an increase from 0.0114 ppb.

Keywords: Residues, pesticides, dates, south Tunisia, maximum residue levels

1. Introduction
Date palm *Phoenix dactylifera* is widely cultivated in the southern oases of Tunisia and represents a good cash crop for many farmers [1]. It is directly or indirectly a source of life and stability. It covers an area of 50,000 ha, which represents 1.11% of the total agricultural area and 18% of the total area of irrigated areas. The total production of dates during 2018 season was 228,400 Tons of variety "Deglet Nour" [2]. The main variety produced in palm groves is the Deglet Noor variety which is highly prized for its nutritional qualities [3]. However, this variety is very sensitive to many diseases and pests such as the carob moth *Ectomyeloïs ceratoniae* Zeller (1839) (Lepidoptera, Pyralidae) [4] and the spider mite *Oligonychus afrasiaticus* (McGregor) (Acari, Tetranychidae) [5] which is considered to be an important injurious Pests of date palm. During many years, the suppression of insects and mites especially *Oligonychus afrasiaticus* in several countries such as Iran, Middle East countries and North Africa relied on calendar broad spectrum applications of synthetic pesticides, such as amitraz, fenpropathrin, fenpyroximate, fenazaquin, propargite, tetradifon, and hexythiazox [6]. Agrochemicals have enabled to more than duplicate food production during the last century, and the current need to increase food production to feed a rapid growing human population maintains pressure on the intensive use of pesticides and fertilizers. However, worldwide surveys have documented the contamination and impact of agrochemical residues in soils, and terrestrial and aquatic ecosystems including coastal marine systems, and their toxic effects on humans and nonhuman biota. Although persistent organic chemicals have been phased out and replaced by more biodegradable chemicals, contamination by legacy residues and recent residues still impacts on the quality of human food, water, and environment [7]. Recently, The Maximum Residue Limits (MRLs) is established by the European Commission Union (EU) and FAO [8, 9]. In fact, the new European legislations forced stakeholders to review control strategies adopted against insects in order to protect the environment and particularly to avoid emergence of resistance towards synthetic pesticides as well as residues in fruits and vegetables proposed to consumer.
In this context, the aim of this present study is to monitor pesticide residues in dates sampled from Tunisian oasis during the agricultural season 2017-2018 and to check compliance with existing regulations especially for European Community.

Material and Methods

Samples and Sampling

The monitoring was conducted during the agricultural season (2017-2018).The sampling concerned the '*Phoenix dactylifera'* fruits, deglet nour variety, collected from 7 oasis respectively located on the main production region of the south of Tunisia. All samples were labeled and stocked at 4°C until the extraction was done.

Chemical pesticides residues

An analysis of pesticides residue of 24 active ingredients used for chemical treatment in fruits was carried out. For analysis, 7 samples of 1kg of palm date (A, B, C, D, E, F, G) were taken from 7 orchards from governorates of Tozeur and Gabes detailed in Table 1.

Table 1: Sites and origin of different samples of palm date (treated and non-treated)

Sites	Origin	Treated/ non treated
A	Dgech	treated
B	Nefta: Amrah Lahwar	Non treated
C	Dgech: Oasis Mahassen	treated
D	Tozeur1	treated
E	Gabes	Non tretaed
F	Tozeur2	Non treated
G	Tozeur3	Non treated

Materials and Reagents

Standards of 24 selected active ingredients: Fenazaquin, Diafenthiuron02, Flufenoxuron, Hexythiazox, Propargite, Chlorpyrifos ethyl, Bifenazate, Clofentezine, Deltamethrine, Spinosad, Spirodiclofen, Spiromesifen, Acrinathrine, (E,Z)-Fenpyroximate, Imidacloprid, Indoxacarbe, Lambda cyhalothrine, Malathion, Methomyl, Methiocarb, Oxamyl, Phosmet, Thiacloprid, Emamectine benzoate were obtained from the stocks of the laboratory of Analysis of Pesticides in the Ministry of Agriculture (Table 2). The individual stock solutions were prepared by dissolving 100 mg of each compound in 100 mL of acetonitrile. Then they were stored in glass stopper bottles at 4 °C.

Table 2: List of active ingredients tested for their residues

	Active ingredients	Formula	Type of pesticide
1	Fenazaquin	$C_{20}H_{22}N_2O$	Acaricide
2	Diafenthiuron02	$C_{23}H_{32}N_2OS$	Insecticide
3	Flufenoxuron	$C_{21}H_{11}ClF_6N_2O_3$	Insecticide
4	Hexythiazox	$C_{17}H_{21}ClN_2O_2S$	Acaricide
5	Propargite	$C_{19}H_{26}O_4S$	Acaricide
6	Chlorpyrifos ethyl	$C_9H_{11}Cl_3NO_3PS$	Insecticide
7	Bifenazate	$C_{17}H_{20}N_2O_3$	Acaricide
8	Clofentezine	$C_{14}H_8Cl_2N_4$	Acaricide
9	Deltamethrine	$C_{22}H_{19}Br_2NO_3$	Insecticide
10	Spinosad	$C_{41}H_{65}NO_{10}$	Insecticide
11	Spirodiclofen	$C_{21}H_{24}Cl_2O_4$	Acaricide/ Insecticide
12	Spiromesifen	$C_{23}H_{30}O_4$	Insecticide
13	Acrinathrine	$C_{26}H_{21}F_6NO_5$	Insecticide
14	(E,Z)-Fenpyroximate	$C_{24}H_{27}N_3O_4$	Acaricide
15	Imidacloprid	$C_9H_{10}ClN_5O_2$	Insecticide
16	Indoxacarbe	$C_{22}H_{17}ClF_3N_3O_7$	Insecticide
17	Lamda cyhalothrine	$C_{23}H_{19}ClF_3NO_3$	Insecticide
18	Malathion	$C_{10}H_{19}O_6PS_2$	Insecticide
19	Methomyl	$C_5H_{10}N_2O_2S$	Insecticide
20	Methiocarb	$C_{11}H_{15}NO_2S$	Insecticide/ Acaricide
21	Oxamyl	$C_7H_{13}N_3O_3S$	Insecticide/ Acaricide/nematicide
22	Phosmet	$C_{11}H_{12}NO_4PS_2$	Insecticide
23	Thiacloprid	$C_{10}H_9ClN_4S$	Insecticide
24	Emamectine benzoate	$C_{56}H_{81}NO_{15}$	Insecticide

Extraction procedure

The extraction procedure is based on the method of QuECHERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) was adopted. This method was recently introduced and is validated to use in the Laboratory of Analysis of Pesticides in the Ministry of Agriculture in Tunisia. It employs dispersive solid phase extraction (SPE) followed by chromatographic analysis of the extracts (10). Liquid Chromatography coupled with tandem Mass Spectrometry was adopted to achieve qualitative and quantitative analysis. The date samples were freezing and blending to get thoroughly homogeneous sample, then 10g from each sample was extracted with 10 mL of acetonitrile; 100µL of internal standard (Tris-[2-chloro-1(chloro-méthyl)-éthyl] phosphate) was added and the sample was shaken for 1 min. Following this step, a mixture of salts: 4g of MgSO4, 1g of NaCl, 1g of trisodium citrate dihydrate and 0.5g disodium hydrogeno citrate sesquihydrate was added to each sample in 50mL tubes. After shaken vigorously the mixture, tubes were then centrifuged for 5 minutes at 3500 U/min to separate solid from acetonitrile supernatant which was ready to dispersive Solid Phase Extraction with 100 mg PSA (Primary and Secondary Amine) and 900 mg MgSO4. Samples collected were coarsely chopped and homogenized for 5 minutes at 3500 U/min at high speed using a laboratory food chopper to separate solid material. The final extract was stabilized by 5% formic acid in acetonitrile solution.

Chromatographic Analysis

Analysis of final extracts stabilized were finally analyzed by LC/MS/MS system equipped with a binary solvent pumps, an autosampler, and a mass-selective detector with Electro-Spray Ionization (ESI) [10] following LC conditions (table 3): mobile phase A: 5mmol.L-1 (ammonium acetate/water, V/V), mobile phase B: 5mmol.L-1(ammonium acetate/-methanol, V/V), stationary phase was a reversed phase silica gel C18 (100mm, 2.1 mm and 1.9µm), adopted flow rate as 0.2 mL min-1, injection volume was 10µL and oven temperature 40 °C. For mass spectrometry conditions, nebulizing gas flow rate was 3Lmin-1, drying gas Flow: 15 L/min, Block heater temperature: 400 °C and ionization mode is the electro spray ionization (ESI).

Table 3: Conditions of the chromatographic analysis.

Chromatographic Liquid (LC)	
Column	Inertsil ODS-3 (L=100mm*2.1mm*3µm)
Mobile phase flow	0.2ml/min
Temperature oven/ column	40°C
Mobile phase	5mmol.L-1 (ammonium acetate/water, V/V) 5mmol.L-1(ammonium acetate/-methanol, V/V)
Injection Volume	10µl
Mass-Spectrometry (MS-MS)	
Nebulizer Gas flow-rate	3L/min
Drying gas flow-rate	15L/min
Vaporizer temperature	400°C
Nebulizer Gas Argon	Argon of 230 KPa

Results and discussion

Residues analysis:

Results showed that the residue concentration of Fenazaquin in the site (A) was 0.0114 ppm and this exceeded the upper

limit of the maximum allowed residue set by either the European Commission Union (EU) and the FAO (LD = 0.1 µg.Kg-1) [8, 9].

Fenazaquin is a non-systemic and specific acaricide/insecticide widely used in controlling mites and other pests infesting fruits and vegetables [11]. In Tunisia, acaricides are used in date palms to control the date mite *O. afrasiaticus*, one of the most important pests of the date palms commonly known 'Boufaroua' [12-14]. The infestation period of *O. afrasiaticus* on fruits vary between years, ranging from the first to the third week of July. From late August, populations of *O. afrasiaticus* decrease with the colour change of fruits to yellow, and remain relatively low for the rest of the season [14]. Sulfur applications are frequently used by Tunisian farmers to control this mite species [14]. Moreover, Fenazaquin 200 g/L (Pride®) is commercialized in Tunisia to control mites on apple, citrus, vegetables and ornementals The Preharvest Interval (PHI) indicated for the fruit trees treatments is 28 days [6]. Despite the use of this 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 [6].

In the other sampled sites, the concentrations of the remaining 23 molecules don't exceed the Limit of Detection (LD). As we know, we are the first to monitor pesticide residues in date palm in Tunisia and to check compliance with existing regulations especially for European Community. Based on the results presented above, it is crucial to think of better supervision of farmers by experts throughout the year and the gathering period in order to moderate chemical treatments and avoid MRL excess Fenzaquin which is very harmful for the environment, non target organisms and especially for human health.

Table 4: List of active ingredients tested for their residues with different techniques of analysis (LMR: Maximum Residue Level, LD: Lethal dose)

Active ingredients	MRL (ppm)	LD(ppm)	Technique analysis	Results (ppm): A	B	C	D	E	F	G
Abamectine	0.01	0.1	LC-MSMS	< LD	< LD	< LD	< LD	< LD	< LD	< LD
Acetamipride	0.01									
Acrinathrine	0.02									
Bifenthrine	0.01		GC ECD							
Clofentezine	0.02		LC-MSMS							
Deltamethrine	0.01									
Emamectine benzoate	0.01									
Fenazaquin	0.01			0.0114						
Fenpyroximate	0.01			< LD						
Flufenoxuron	0.05									
Hexythiazox	2									
Imidaclopride	0.05									
Malathion	0.02									
Methomyl	0.01									
Methiocarb	0.2									
Phosmet	0.05									
Propargite	0.01									
Spinosad	0.02									
Spirodiclofen	0.02									
Spiromesifen	0.02									
Thiacloprid	0.01									
Tetradifon	0.01		GC ECD							

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