# Pesticide residues in date palm and olive oil samples produced and consumed in Jordan

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## Abstract

The presence of pesticides in fruits and vegetables is becoming a growing concern not only for consumer but also for growers, processors and competent authorities in the entire world. In Jordan, there are only four specialized laboratories which have the required infrastructure to accurately determine pesticide residues in agricultural products. The aim of this study was to investigate the presence of PPPs residues in nationally produced palm dates and olive oil for local consumption and exportation. Among the283 pesticides analyzed, only 7 were detected in palm dates at concentrations ranging from detectable/quantifiable below the MRLs (4 µg/kg for carbendazim) to values above the MRLs attaining (508 µg/kg for carbendazim). Regarding olive oil, only 4 were detected at concentrations ranging from detectable below MRLs (20 µg/kg) for cypernethrin to higher than MRLs (260 µg/kg) for chloropyrifos. The order of occurrence for PPPs in date samples was from the greatest to the lowest as follows: carbendazim (53.33%), hexythiasox (21.3%), spiromesifen (12%), thiacloprid and finazinan (2.6%), thiophanate-methyl (6.6%), and imidacloprid (1.3%) and was in olive oil as follows: chloropyrifos (27.45%), cypermethrin (25.49%) and finally both oxyfluorfen and myclobutanil (3.92%). 84% of the date samples had pesticide residue lower than MRLs and only 16% had residue higher than MRLs. However, in olive oil samples, 49.01% had residue below the MRLs, 9.8% were above the MRLs and interestingly in 41.17% had no detected pesticide residues. The main part of the palm date tested samples (80.32%) were containing simple pesticide residue and (19.67%) exhibited double pesticide residue. However, in olive oil samples, only one of the reported pesticides was detected. Although the limited biological diversity (just one-year samples), we suggest, the urgent implementation of programs designed to facilitate awareness about the health concerns from PPPs and programming a compulsory and periodical training sessions for specialized workers and traders in Jordan.

***Key Words:*** *Pesticide residues, date palm, olive oil, MRLs*

## Introduction

The production of palm date and olive oil is considered as a strategic and primary sector for the Jordanian economy. In fact, in 2017, the Jordanian kingdom produced 25 222 tons of palm date fruits and 14 5073 tons of olive for both local and international markets (FAO Stat, 2019).

Fruits and vegetables consumption are increasing in the modern life style due to their content of natural antioxidants (Ilahy et al., 2018, 2020, Siddiqui et al., 2018). However, the improper use of PPPs (Plant Protection Products) might lead to increased risk of consumer health following high fruit and vegetables intake.

PPPs are also known as pesticides that protect crops or desirable or useful plants mainly used in the agricultural sector but also in forestry, horticulture, and in-home gardens. The PPPs contain at least one active substance and have numerous functions (European Commission for Food Safety, 2019).

Countries over the world are increasingly implementing new strategies to address improper PPPs usage and increase the food feed inspections and therefore safety along the exportation of agricultural commodities by as control laboratories and competent specialized authorities. Although the economic importance of palm date and olive oil sectors, palm date and olive oil trading are becoming increasingly hampered by MRLs (Maximum Residue Levels) exceedance for producing countries, including Jordan. Therefore, concerns are increasingly arisen in order to protect both local and international consumers. Previously, Algharibeh and AlFararjeh (2019) focused on pesticide residues in various fruits and vegetables gron and produced in Jordan using liquid chromatography/tandem mass spectrometry. However, palm date and olive oil were undertaken in this study although their primary importance for consumption and export traffic in Jordan.

Based on the above mentioned, the aim of this study was to perform a sampling of the main agricultural products in Jordan (palm date fruits and olive oil samples) from the main producing zones in the Kingdom and screening for the presence of 238 different pesticide active substance with respect to their MRLs using both LC-MS/MS and

GC-MS/MS analytical tools. This screening will be useful for suggesting urgent procedure or National plan to limit the excessive and the issue of plant protection products active ingredients. In addition, recent EU regulations (Sustainable Pesticide Use Directive) and principles in the document are discussed and considered in favour of improving palm oil and date consumers safety.

## Materials and Methods

*Laboratory working procedure*
In 2018, the Jordanian Food and Drug Administration, performed a general check-up of various agricultural commodities for the presence of PPPs residues. For this purpose, 61 palm date fruits samples were gathered from different palm date producers in the Jordan Valley. The samples were accurately coded and delivered to the laboratory for further analysis to be screened for the presence of at least 283 pesticide active ingredient.
*Solvents and reagents*
The products used were all LC-MS grade Acetonitrile (MeCN), Toluene, 5% Formic acid solution in acetonitrile, Water, Methanol, Ammonium formate and Formic acid. Pesticide standards were purchased from Sigma Aldrich.
*Sample preparation*
Ready oil samples need no preparation and were stored immediately after reception and put in freezer at-20℃ or colder until analysis. However, for palm date, samples were weighed and the equivalent amount of LC-MS grade water was added then thoroughly homogenized and stored at -20℃ or colder and analyzed subsequently
*Extraction procedure*
Initially, 5 g of olive sample were weighed into a clean 50 mL tube. The blank samples were spiked at concentration 0.05 mg/kg and let further to stand for 10 minutes. Then 10 mL acetonitrile were added, and the mixture was vortexed for 1 min. The obtained extract was centrifuged at 5°C for 5 minutes at 6000 rpm then an aliquot of 6 mL of the acetonitrile phase (upper layer) was transferred into a 15 mL screw capped tube and placed at -20 ℃ or colder at least for 2 hours then the extract was centrifuge at 5°C for 5 minutes at 4000 rpm.

*Clean-up procedure*
For the clean-up procedure, 2 mL of the extract were filtered through 0.45 mm. For LC-MS/MS, a sample of 0.5 mL of the filtrate was acidified with 5% formic acid in acetonitrile (10 µL to every 1 mL). Then in in lab solutions software, the sample amount was set at 0.5 and the dilution at 1 so that the final extract is a concentration of ca. 0.5 g/mL.
Regarding GC-MS/MS a sample of about 0.5 mL of the filtrate was evaporated to almost dryness at 40 °C using a slight nitrogen flow (less than 5 bars) and reconstituted in 0.5 mL of toluene and mixed properly. The mixture was then acidified with formic acid 5% in acetonitrile (10 µL to every 1 mL) and in the Trace Finder software, conversion factor was set at 1 so that the final extract is a concentration of ca. 0.5 g/mL and then injected.

*Analysis technique*
Liquid Chromatography coupled with Mass Spectrometry-Mass spectrometry (LC-MS-MS)
HPLC-MS analyses were carried out using a Nexera (2040) LC system, Shimadzu. The chromatographic part was a Shimadzu 2040C chromatograph equipped with a Restek Raptor Biphenyl (100, 2.1mm, 2.7mm). The solvent A consisted of 2mmol/L ammonium formate + 0.002% formic acid-water mixture and the solvent B consisted of 2mmol/L ammonium formate + 0.002% formic acid methanol mixture. The volume injected by the automatic sampler/injector was 2µl with a flow rate of 0.4 ml/min under 35°C of column oven temperature. The gradient program started with 97% (A) and 3% (B) to attain in the middle of the run 0% (A) and 100% (B) and finish in the same conditions as initially indicated.
Regarding the MS conditions, the instrument used was an LC8050 system, Shimadzu with a nebulizing gas flow rate of 3L/min under an interface temperature of 350°C. The ionization mode was (ESI) and both the drying and heating gas flow rate was 10L/min.

*Gas Chromatography coupled with Mass Spectrometry-Mass spectrometry (GC-MS-MS).*
After extraction of pesticides that could be analyzed by gas chromatography were identified by GC-MS-MS coupling (Cheng et al., 2017). The chromatograph system was composed of a Trace 1310 Gas Chromatograph (Thermo Fisher Scientific- USA) equipped with TSQTM
Duo Triple Quadrupole Mass Spectrometer (Thermo Fisher Scientific- USA). Pesticides were separated with an RTX-5MS (30 m, 0.25 mm ID, 0.25µm) under the following conditions:
- Injector temperature: 250°C

- Split less injection: 2µl
- Column temperature: 90°C (hold 1min) 330°C at 8.5°C/min (hold 5 min)
- Carrier das: He (1.4 ml/min)

Regarding the MS conditions, the instrument used was an LC8050 system, Shimadzu with a nebulizing gas flow rate of 3L/min under an interface temperature of 350°C. The ionization mode was (ESI) and both the drying and heating gas flow rate was 10L/min. The results should always be reported and expressed in µg/kg (El-Saeid & AL-Dosari, 2010; Ferrer et al.,2005).

## Results and Discussion

***Date samples***

As described in the Materials and Methods chapter, in 2018, the Jordanian Food and Drug Administration performed a general check-up of various agricultural commodities for the presence of PPPs residues. For this purpose, 61 palm date samples were gathered from different palm date producers in the Jordan Valley and 51 olive oil samples were gathered from olive oil producers in the region of Irbid located in the North of the Kingdom. The samples were accurately coded and delivered to the laboratory for further analysis to be screened for the presence of at least 283 pesticide active ingredients. Among the 283 pesticides that we have analyzed, only 7 were detected in palm date samples at concentrations ranging from detectable/quantifiable below the MRLs (Table 1) (4 µg/kg for carbendazim) to values above the MRLs attaining (508 µg/kg for carbendazim).

**Table 1: LOQ and MRLs for different pesticide active substances detected in palm date fruit sample**

| Agricultural crop | PPPs active substance | LOQ (ppm) | MRLs (ppm) |
|---|---|---|---|
| Date palm fruit samples | carbendazim | 0.05 | 0.1 |
| | hexythiasox | 0.093 | 2 |
| | spiromesifen | 0.003 | 0.02 |
| | finazinam | 0.02 | 0.01 |
| | thiacloprid | 0.01 | 0.01 |
| | thiophanate-methyl | 0.003 | 0.1 |
| | imidacloprid | 0.05 | 0.05 |

All the samples tested were positive for the presence of the residue for at least one active substance among the list of the following seven: carbendazim, hexythiasox, spiromesifen, thiacloprid, thiophanate-methyl, finazinam and imidacloprid). The other pesticides were not detected in all the palm date samples. However, it does not confirm complete freedom from pesticides since the other might be present but under instrumental detection (with respect to the used detection equipment).

The percentage of residues occurrence varied among the tested pesticide (Figure 1). In fact, the order of occurrence was from the greatest to the lowest as follows: carbendazim (53.33%), hexythiasox (21.3%), spiromesifen (12%), thiacloprid and finazinan (2.6%), thiophanate-methyl (6.6%), and imidacloprid (1.3%).

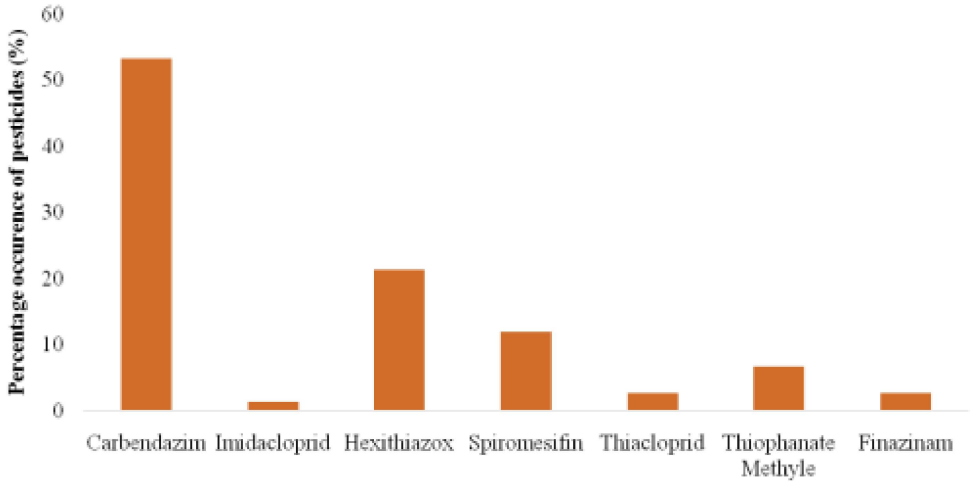


**Figure 1: Percentage of occurrence of different detected pesticide active substances in date samples** (2018, Jordan valley/ Random duplicate sample in each batch (20 samples/batch)

Among the palm date tested samples, 61 were positive, but also divided in samples with single and other with multiple pesticide residues. In our study, the main part of the tested samples (80.32%) were containing simple pesticide residue and others (19.67%) were samples with double pesticide residue (Figure 2). The result does not also confirm the absence of other pesticides in the tested samples since their presence might be not detected as hampered by instrumental LOD.

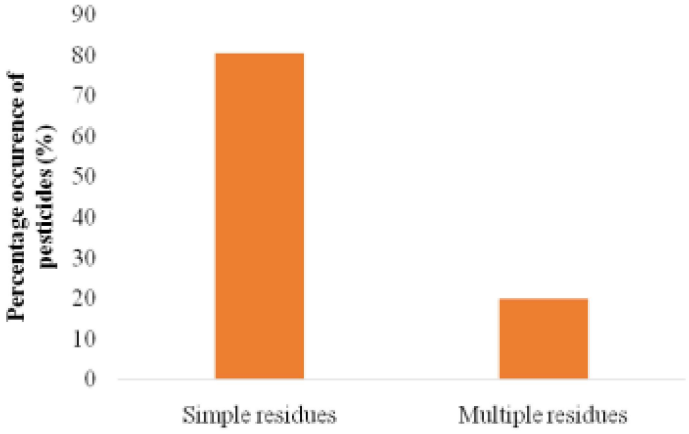


**Figure 2: Percentage of occurrence of simple and multiple residues in date samples**

Regarding the Maximum Residue Level, commonly known as MRLs, when data from all the palm date samples was considered, 84% of the samples had pesticide residue lower than MRLs and only 16% had residue higher than MRLs (Figure 3). Our values are similar to those of EL-Saeid and AL-Dosari (2010) who used different techniques for the analysis of various insecticide, fungicide, acaricide, and herbicide residues in three cultivars' of date fruits namely, Khalas, Sukkari, Nabout Seif as well their seeds. The authors reported that carbendazim was detected in samples of the three cultivars at 0.05, 0.04, and 0.03 ppm. Respectively, the residue level of cypermethrin in Khalas cultivars was 0.02 ppm (MRL 0.1 ppm). Cypermethrin was not detected in Sukkari cultivar but was detected in Nabout Seif at 0.03 ppm (MRL 0.01 ppm).

Additionally, the residue levels of both insecticides dimethoate and chlorpyrifos found in all the three sample date cultivars Khalas, Sukkari and Nabout Seif were higher three times compared with MRLs. Nevertheless, in this study also, glyphosate was detected in all three cultivars at the following levels 0.02, 0.02, and 0.03 ppm, respectively. The

fungicide benomyl was detected in Khalas date samples only, and the residue level was 0.04 ppm. Carbendazim was detected in the three cultivars Khalas, Sukkari, and Nabout Seif (0.05, 0.05, and 0.03 ppm, respectively. Benomyl was detected in Khalas date samples (0.02 ppm).

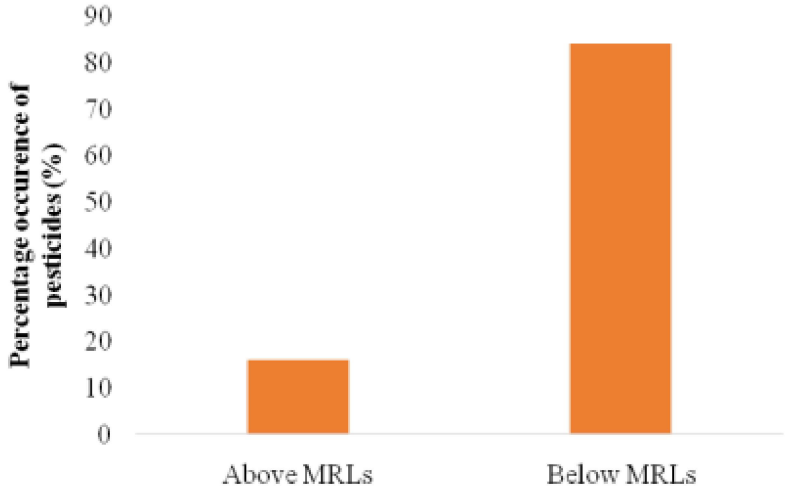


**Figure 3: Percentage date samples below or above MRLs**

***Olive oil samples***

A total of 51 olive oil samples were gathered from olive oil producers in the region of Irbid located in the North of the Kingdom. The samples were accurately coded and delivered to the laboratory for further analysis to be screened for the presence of at least 283 pesticide active ingredients.
Among the 283 pesticides tested, only 4 were detected at concentrations ranging from detectable below MRLs (20 µg/kg) for cypernethrin to higher than MRLs (Table 2) (260 µg/kg) for chloropyrifos.

**Table 2: LOQ and MRLs for different pesticide active substances detected in olive sample**

| Agricultural crop | PPPs active substance | LOD (ppm) | MRLs (ppm) |
|---|---|---|---|
| Olive oil samples | chlorpyrifos | 0.01 | 0.25 |
| | cypermethrin | 0.01 | 0.25 |
| | oxyfluorfen | 0.02 | 5 |
| | myclobutanil | 0.01 | 0.1 |

The pesticide residue was not detected in all olive oil samples. In fact, 49.01% of the samples had residue below the MRLs, 9.8% were above the MRLs. Interestingly, in 41.17% of the tested olive oil samples, no pesticide was detected. However, it does not confirm totally the absence of pesticide residues since other might be present but under LOD for the used analytical procedure (Figure 4)

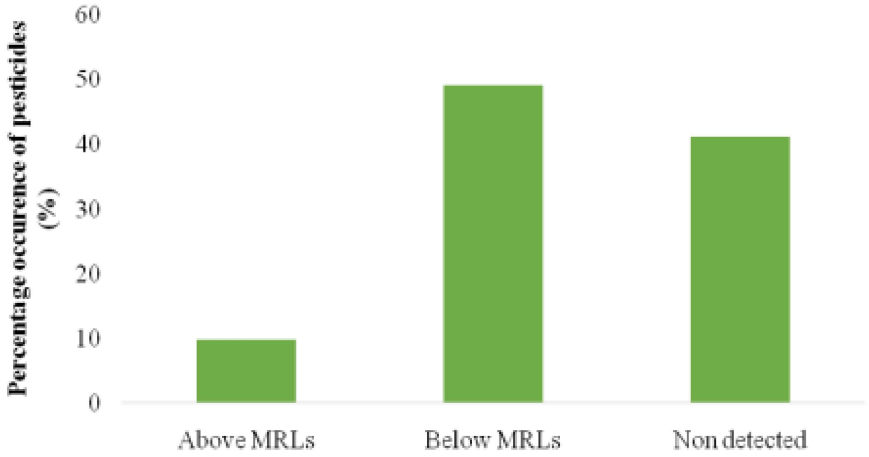


**Figure 4: Percentage olive oil samples below or above MRLs and free pesticides samples**

The percentage of residues occurrence varied among the 4 pesticide (Figure 5). In fact, the order of occurrence was from the greatest to the lowest as follows: chloropyrifos (27.45%), cypermethrin (25.49%) and finally both oxyfluorfen and myclobutanil (3.92%).

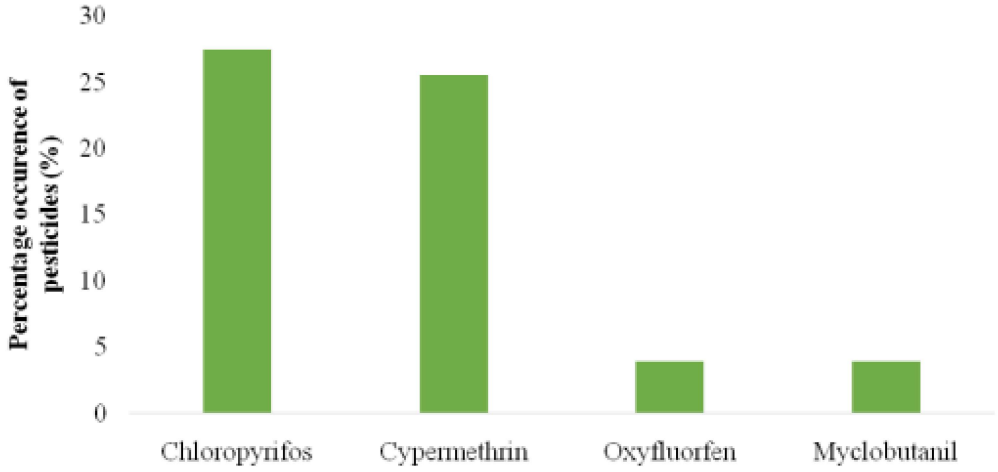


**Figure 5: Percentage of occurrence of different detected pesticides active substances in olive oil samples (Irbid/ Random duplicate sample in each batch (20 samples/batch)**

Our results (20-80 microgram/kg fw for cypermethrin) were close to the lowest value reported by Ferrer et al. (2005) who focused on the occurrence of various widely used pesticides (dimethoate, simazine, atrazine, diuron, terbuthylazine, methyl-parathion, methyl-pirimiphos, endosulfan I, endosulfan II, endosulfansulphate, cypermethrin and deltamethrin), which can be found at trace levels in olive oil and olives and detected cypermethrin concentrations ranging between 25–500 microgram/kg fw. (One should notice that the vaste majority of the above listed active substances are not registered (anymore) on the EU market). Regarding chloropyrifos, Likudis et al., (2014) reported a mean value for this pesticide attaining 32.9 microgram/kg fw which was also in the range of the values (50-650 microgram/Kg fw) reported in this study.

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