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Sensitive and Rapid Method to Estimate Residual Pesticides in Some Local and Imported Apple Cultivars Collected from Eastern North Side of Libya

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Abstract

In this study, residues of some pesticides of (Imidacloprid, Aldicarb, metalaxy, cypermethrin, chlorpyrifos, DDA and Endrin) were detected in the two cultivars (Starking and GoIden delicious). The samples were collected from some farms in eastern north of Libya (Shahat, Wardamah and Al-Wasita). Samples of imported apples (Tunisian, Italian and Turkish) were collected from the local supermarkets. The level of pesticide in the apple samples under study were compared with the international permissible limits and according to the specifications. An ultraviolet-visible (UV-Vis) spectrophotometer, rapid, sensitive, efficient, and safe purification extraction method (QUECERS) were used for the determination of pesticides present in apple samples. The limits of detection and quantification of the (UV-Vis) instrument ranged in standard pesticides, where the limits values for cypermethrin were 0.52 and 1.58 µg/ml, chlorpyrifos 0.53 and 1.61 µg/ml, and Imidacloprid 0.64 and 1.95 µg/ml. metalaxyl between 0.27 and 8.32 µg/ml, Aldicarb 0.90 and 2.73 µg/ml, DDA 3.50 and 10.62 µg/ml, 2.74 and 8.32 µg/ml and Endrin 1.89 and 5.73 µg/ml, respectively. The results indicated the presence of residual Chlorpyrifos in some varieties of apple samples. The pesticide residues tested were found to be below safe limits.

Keywords: Organic Pesticides, UV/Vis Spectroscopy, Residual Effect of the Pesticide

Introduction

The apple (Malus domestica) is an important temperate fruit with commercial value. It is the fourth most produced fruit in the world after bananas, oranges and grapes. Apples are known for their nutritional and health benefits. It is a good source of dietary fiber, minerals, vitamin C, and many other antioxidants. Apple peel is rich in several health-promoting agents such as anticarcinogenic, anti-inflammatory, and vascular protective (Ben-Yehudah *et al*., 2005) [4]. Apple juice has a rich nutritional profile and is often included in the diet (Xu *et al*., 2018; Ullah *et al*., 2018) [22, 20]. Among the best varieties of apples are apple varieties that are round and round, with elastic and smooth skin. The core of the apple is firm, usually harvested in late September or early November (Mohebi *et al*., 2017; Dana *et al*., 2018) [13, 6]. Apples are a widely cultivated fruit tree and economic tree in temperate regions of the world (Fan *et al*., 2014) [8]. To combat various apple pests and diseases, farmers use a number of pesticides. However, the use of pesticides and unrecommended doses leads to pesticide residues in the fruits. Although humans are exposed to small amounts of pesticide residues after the pesticides are metabolized by plants or degraded by environmental factors, small amounts of pesticides persisting in the human body for long periods can cause chronic diseases such as cancer (Carozza *et al*., 2009) [5]. As a result, food commodities are often found with unsafe levels of pesticides due to unwise use and non-compliance with post-harvest periods. Residues of pesticides such as chlorpyrifos and cypermethrin have occasionally been detected in apples at levels exceeding maximum residue limits (MRLs) (Shukla *et al*, 2021) [17]. Apple production is one of the crops most susceptible to damage by a number of pests that cause serious problems for different crops (Kutinkova *et al*., 2006) [10].

In addition, there is a growing concern due to a lack of awareness among consumers about the potential harmful effects of unsafe levels of pesticide residues in fruits and vegetables, and knowing how to reduce pesticide residues in produce to safer levels (Lu *et al*., 2013). Research results in Libya indicated the presence of residues of chlorine, phosphorus and carbamate pesticides in some vegetables and fruits (Al-Jarari, 2015; Mohammed, 2014) [16, 1].

Materials and Methods

Sampling

Different six apple samples were collected in both varieties (Starking and GoIden delicious) from the farms of (Shahat, Wardamah and Al-Wasita) regions during the end of August and the beginning of September. Also, six markets were randomly selected from the city of Al-Bayda (Tunisian, Italian and Turkish). About 500 grams were collected from each of two types (Starking and GoIden delicious). The name of the region and the date of sampling were written on it, then the samples were transferred to a refrigerated container directly to the laboratory, and the collected apple samples were stored in a refrigerator at a temperature of 4 °C until they were analyzed (Hunt and Wilson, 1986; APHA, 1995) [9, 2].

The Chemicals

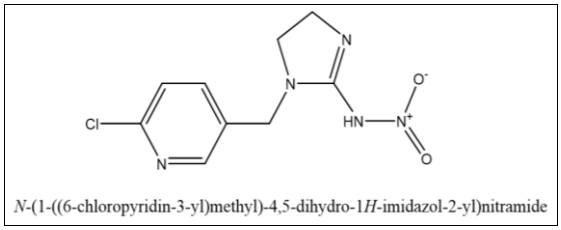
Seven standard pesticides were used (Imidacloprid, Aldicarb, metalaxyl, cypermethrin, chlorpyrifos, DDA, and Endrin). The purity of the standard pesticides was 99% from Augsburg, Germany) and acetonitol 99.9%-PSA (primary secondary amine) 99.9%-sodium sulfate anhydrous 90%–sodium chloride 90% from BDH (UK)

Selection of pesticides:

Most of the common pesticides as well as pesticides that are banned in the world were included in this study based on their presence in the samples and the cases of high maximum residue limits of the studied pesticides.

1. Imidacloprid

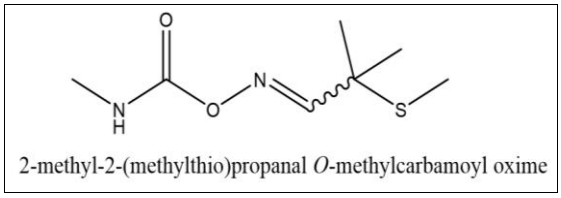
Trade name: Snapper



N-(1-((6-chloropyridin-3-yl)methyl)-4,5-dihydro-1*H*-imidazol-2-yl)nitramide

2. Aldicarb

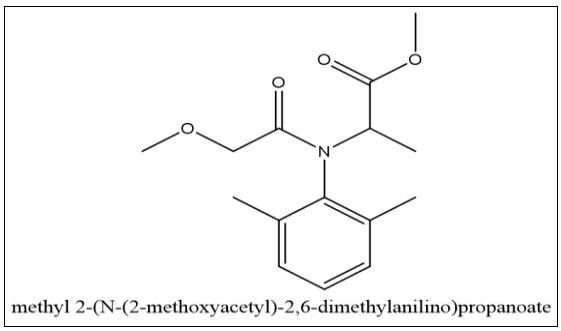
Trade name: Temic



2-methyl-2-(methylthio)propanal *O*-methylcarbamoyl oxime

3. Metalaxyl

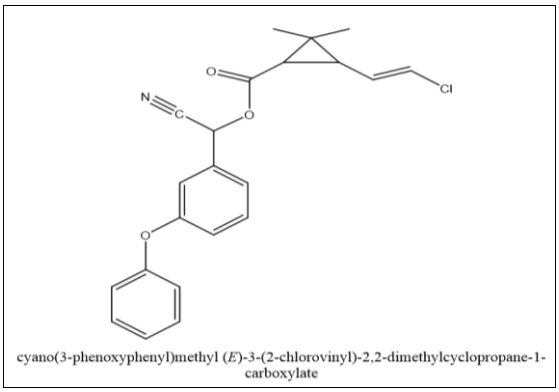
Trade name: Acetamore



methyl 2-(N-(2-methoxyacetyl)-2,6-dimethylanilino)propanoate

4. Cypermethrin

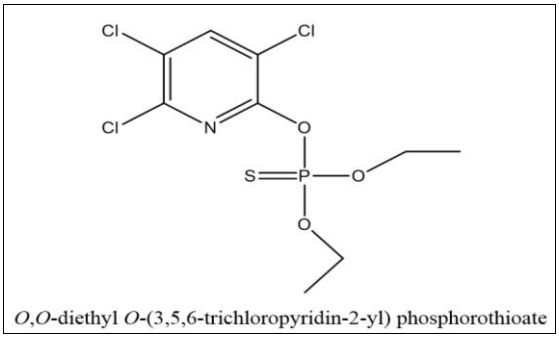
Trade name: Cyberkill



cyano(3-phenoxyphenyl)methyl (*E*)-3-(2-chlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate

5. Chlorpyrifos

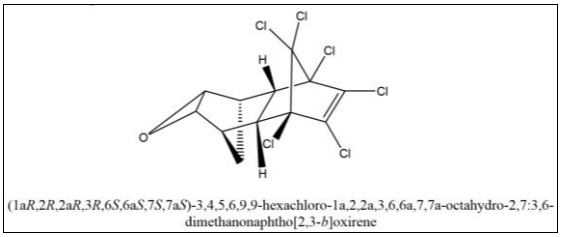
Trade name: Durspan



O,*O*-diethyl *O*-(3,5,6-trichloropyridin-2-yl) phosphorothioate

6. Endrin

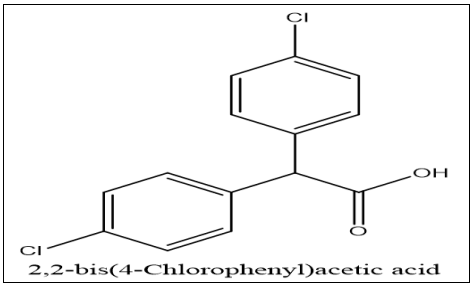
Trade name: Compound 269



(1a*R*,2*R*,2a*R*,3*R*,6*S*,6a*S*,7*S*,7a*S*)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphtho[2,3-*b*]oxirene

7. DDA

Trade name: Gesarol



2,2-bis(4-Chlorophenyl)acetic acid

Apparatus used for Determination of Pesticides

All spectral measurements were performed using a dual-beam UV/Vis spectrophotometer (Biotech Engineering Ltd., UK) Thermo Scientific, UK, with wavelength range 190-1100 nm, spectral bandwidth 2.0 nm, with a scan speed of 400 nm /min, and a 1-cm quartz cell was used for all spectroscopy.

Preparation of Standard UV/Vis Pesticides

5 mg of each pesticide was accurately weighed and dissolved in 50 ml of acetonitrile in a standard flask and we had 100 µg/ml diluted to obtain a series of concentrations to give two titration curves with different concentrations in each pesticide.

Extraction

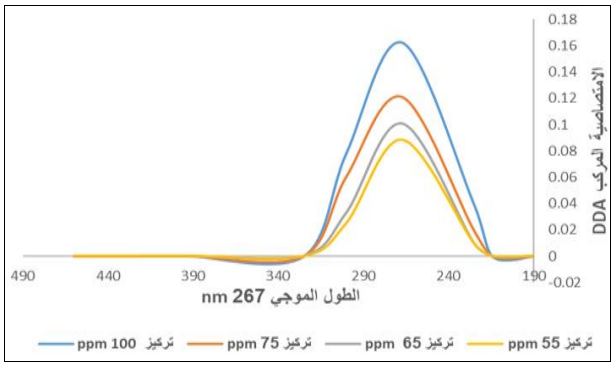
Apple samples were extracted and quantified using the Quechers method (Prodhan *et al*., 2016) [15]. Apple samples were chopped using a home fruit blender. Approximately 10 g of the homogenized sample was transferred to a 50 ml centrifuge tube followed by the addition of 10 ml of acetonitrile and by the addition of (4 g of anhydrous magnesium sulfate + 1 g of sodium chloride) and placed in a shaker for 30 seconds and then in a centrifuge at 5000 rpm for 5 minutes. 3 mL of the liquid was transferred to a 15 mL centrifuge tube containing 600 mg anhydrous magnesium sulfate and 120 mg PSA. They were placed in a shaker for 30 seconds, followed by centrifugation at 5,000 rpm for 5 minutes. Finally, it was transferred to clean quartz for UV/Vis measurement (Begum *et al*., 2019) [3].

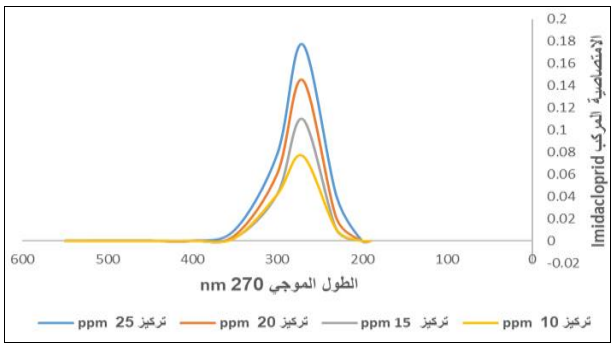
Calculation of Pesticide Concentration in Apple Samples

The concentration of the sample containing the pesticide was calculated by the equation of the straight line for the calibration curves of each pesticide. After obtaining the final concentration of the pesticide, it was divided by the volume from which the sample was extracted to obtain the actual concentration of the pesticide.

Prepare the Calibration Chart

The absorbance of each standard solution was measured with a maximum absorbance of 288 nm using a quartz cuvette. Absorbance values were then plotted against the concentrations to generate a standard calibration curve. Fig 3 shows the spectrum of standard pesticides at different concentrations at a wavelength ranging from 219 to 288 nm.





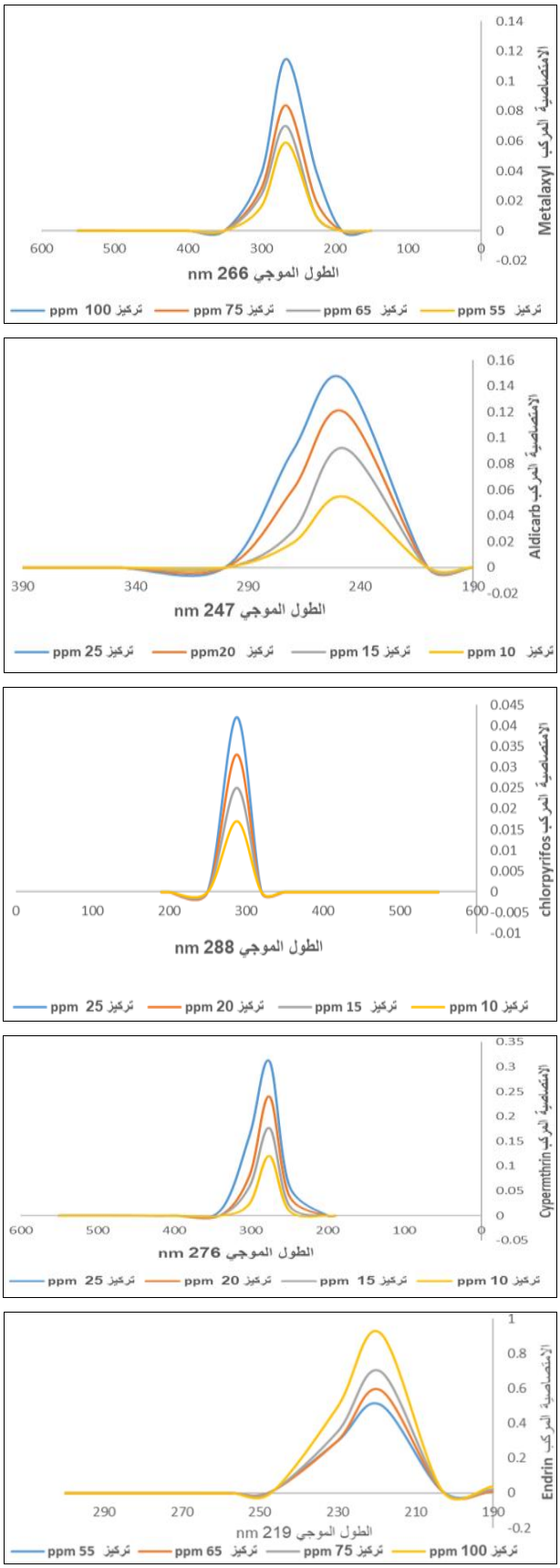
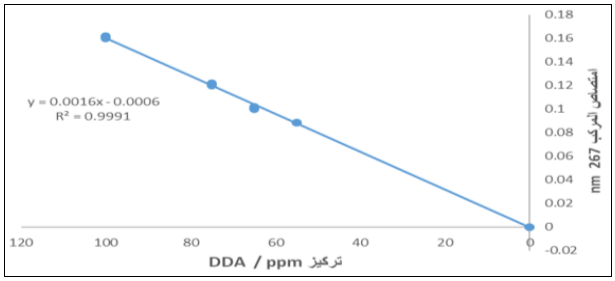
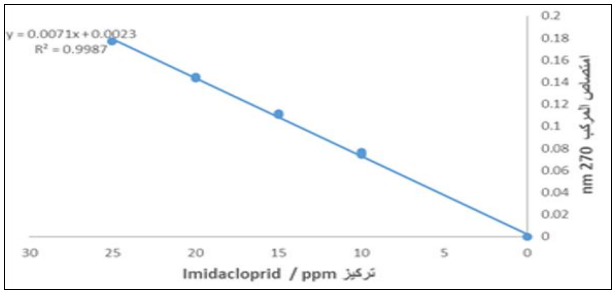


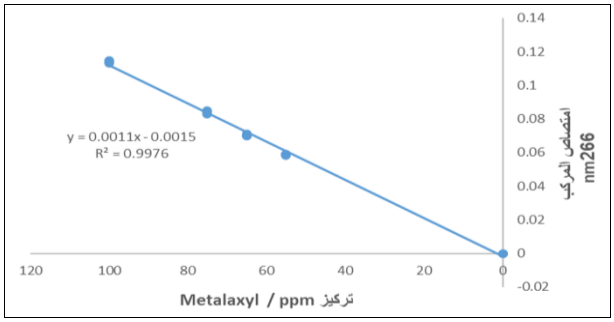
Fig 3: Spectrum of different pesticides

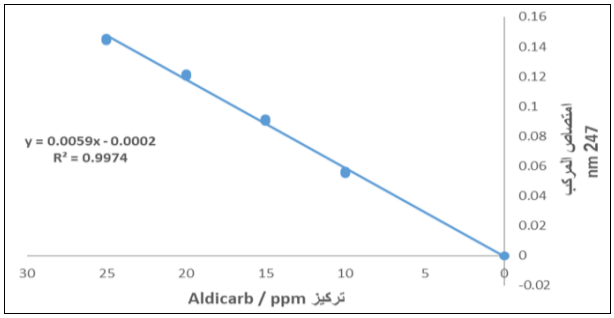
Pesticide Standard Curves

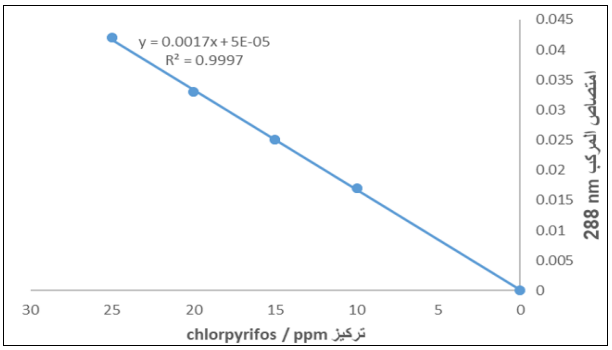
These curves showed the linearity of the method for each pesticide separately, as shown in Fig 4 showed a good linear relationship between absorbance and concentrations of standard solutions.

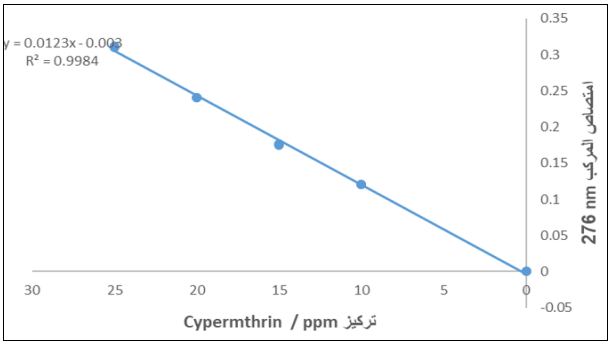












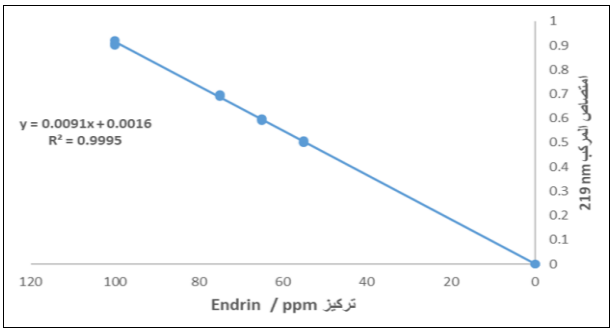


Fig 4: Shows the concentration of each pesticide with the area of the curve corresponding to each concentration

Detection and Quantification Limits of the UV Device

The detection and quantitative limits were calculated for the following pesticides. The values for cypermethrin were between 0.52 and 1.58 µg/ml, chlorpyrifos 0.53 and 1.61 µg/ml, Imidacloprid 0.64 and 1.95 µg/ml, and metalaxy between 0.27 and 8.32. µg/ml, Aldicarb 0.90 and 2.73 µg/ml, DDA 3.50 and 10.62 µg/ml, and Endrin 1.89 and 5.73 µg/ml, respectively, as shown in Table (1).

Table 1: Limits of detection and quantification of standard pesticides for U

S. No.	standard pesticides µg ml $^{-1}$	detection limit (LOD)	Quantitative limit (LOQ)
1.	cypermethrin	0.52	1.58
2.	Chlorpyrifos	0.53	1.61
3.	Imidacloprid	0.64	1.95
4.	Metalaxyl	0.27	8.32
5.	Aldicarb	0.90	2.73
6.	DDA	3.50	10.62
7.	Endrin	1.89	5.73

Statistical Analysis

Data were analyzed using Minitab version 19. The two apple cultivars and the pesticides used in different locations were analyzed using two way ANOVA at a significant difference less than < 0.05. A t-test was conducted to find out the significant differences between the two cultivars, as well as between the local and the imported at less than 0.05.

Results and Discussion

The results indicated that residual chlorpyrifos was found in most of the study areas. Where the pesticide residue was recorded in Shahat in all the two cultivars was 0.064-0.18g/ml µ respectively, as well as the pesticide residue was recorded in the Starking class (0.14) g/ml µ. The pesticide residue in the intermediate was also recorded in both classes 0.17-0.24 g/ml µ, as well as in the records of the pesticide residue chlorpyrifos imported in Turkey only in the class Starking (0.20 (g/ml µ), where the values were below the internationally permissible limits according to the European Union of 0.860 g/ml µ (Witczak *et al*., 2018) [21] Also, (Imidacloprid, Aldicarb, metalalaxy, cypermethrin DDA and Endrin) were not found on any of these pesticides, according to the limits of device detection (LOD) in any sample of apple samples in all the two varieties, except for the residual Chlorpyrifos pesticide.

Table 2: Study samples in some different regions

S. No	µg ml $^{-1}$ study samples		cypermethrin	Chlorpyrifos	Imidacloprid	Metalaxyl	Aldicarb	DDA	Endrin
1.	Shahat	GoIden delicious	ND	0.064	ND	ND	ND	ND	ND
		Starking	ND	0.18	ND	ND	ND	ND	ND
2.	Wardama	GoIden delicious	ND	ND	ND	ND	ND	ND	ND
		Starking	ND	0.14	ND	ND	ND	ND	ND
3.	Al Wasita	GoIden delicious	ND	0.17	ND	ND	ND	ND	ND
		Starking	ND	0.24	ND	ND	ND	ND	ND
4.	Tunisian	GoIden delicious	ND	ND	ND	ND	ND	ND	ND
		Starking	ND	ND	ND	ND	ND	ND	ND
5.	Italian	GoIden delicious	ND	ND	ND	ND	ND	ND	ND
		Starking	ND	ND	ND	ND	ND	ND	ND
6.	Turkish	GoIden delicious	ND	ND	ND	ND	ND	ND	ND
		Starking	ND	0.20	ND	ND	ND	ND	ND

Non Detected

As shown in Table (3), there are statistically significant differences between the study areas in the two classes for all the results that were collected from the study areas. Within the internationally permitted limits.

In a similar study of results by (EL-AWAMI *et al*., 2015) [7], the pesticide concentration was below the maximum residue limits (MRL). As shown in Table (3), the analysis of variance for all results collected from the study regions, the apple samples in both cultivars showed significant differences between the study regions and the two apple cultivars. From these results, we can conclude that organophosphorous insecticide residues were more significant in vegetables and fruits during spring and summer, when insect populations are more active, which leads to more spraying of different insecticides.

Table 3: Analysis of variance between study samples using the LSD test

S. No	Study Samples		Chlorpyrifos/µg ml $^{-1}$	
1.	Shahat	GoIden delicious	0.064	f
		Starking	***0.18***	c
2.	Wardama	GoIden delicious	ND	
		Starking	***0.14***	e
3.	Al Wasita	GoIden delicious	***0.17***	d
		Starking	***0.24***	a
4.	Tunisian	GoIden delicious	ND	
		Starking	ND	
5.	Italian	GoIden delicious	ND	
		Starking	ND	
6.	Turkish	GoIden delicious	ND	
		Starking	***0.20***	b

In general, Fig 5 shows residual concentration of chlorpyrifos in different samples using analysis of variance, and there were statistically significant differences between different samples at a significant difference ($p < 0.05$). The t-test analysis with chlorpyrifos residue showed that there was a significant difference between the two cultivars (GoIden delicious and Starking) in the studied areas at ($p < 0.05$), as shown in Fig 6. From these results, we can conclude that the pesticide residue is more in the Starking variety than in the GoIden delicious variety, due to the lack of a small amount of thiocyanates in the Starking variety, thus leading to an increase in the concentration of pesticides (Pojer *et al*., 2013; Mannino *et al*., 2020) [14, 12]. The residual concentration of chlorpyrifos showed significant differences between local and imported apples in the study areas at ($p < 0.05$), as shown in Fig 7.

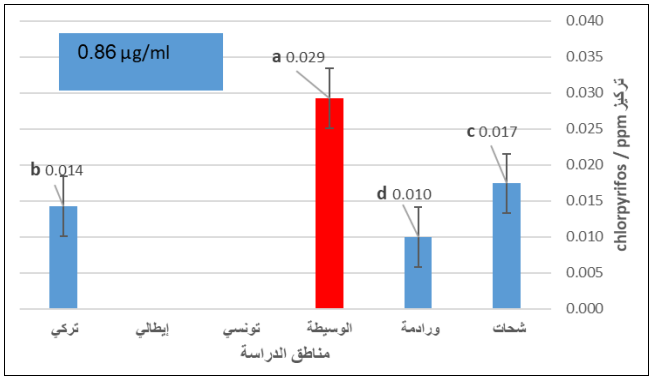


Fig 5: Analysis of the variation of chlorpyrifos residual concentration in the two apple cultivars during the study regions

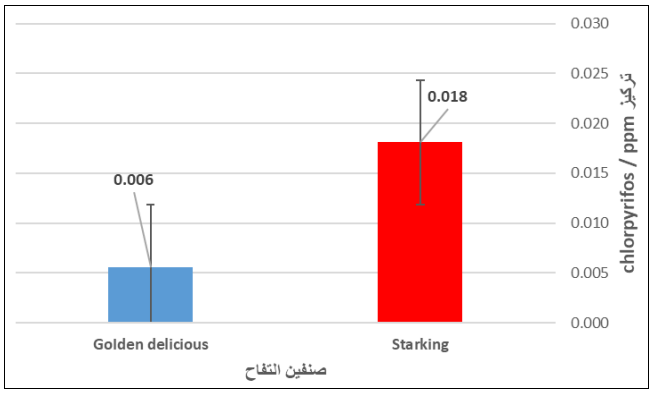


Fig 6: Analysis of the variance of chlorpyrifos residual concentration between the two varieties (GoIden delicious and Starking) using the t-test

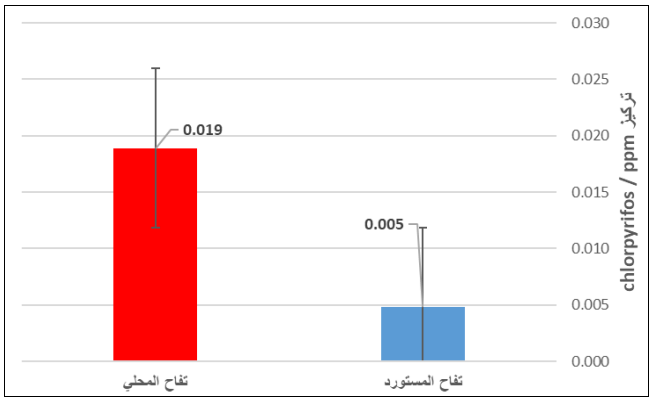


Fig 7: Analysis of the variance of chlorpyrifos concentration between the two imported and local apple cultivars using the t-test

Conclusion

None of the aforementioned pesticides (Imidacloprid, Aldicarb, metalaxyl cypermethrin, DDA and Endrin) were found in this study except for Chloropyrifos pesticide residue in two apple samples in some study areas. It was found that the Starking variety is more contaminated than GoIden delicious with pesticide residue and the highest percentage appears. Pollution in intermediate, followed by scarcity, and the values were less than the permissible limit according to the European Union. The study showed the results of the residual concentration of Chloropyrifos pesticide in the local more than in the imported and due to the phosphorus pesticides are the most widely used in the world on vegetable and fruit crops because of its high efficiency against insects. However, its residues represent a potential threat to public health, so from a health point of view, it is necessary to control and control the application of pesticides to various crops in Libya.

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