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Pesticide Residues Monitoring of Organophosphorus and Carbamates in Grapes in Three Egyptian Governorates

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Authors' contributions

This work was carried out in collaboration between all authors. Author MMA conceived, designed the research and wrote the manuscript. All authors conducted the experiments, analyzed the data, reviewed, read and approved the manuscript.

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ABSTRACT

To assess the level of pesticide residues of 21 organophosphorus (OPs) and 5 carbamates (Carbs) in grapes, a total number of 96 samples of grape fruits and leaves was purchased from the markets of Qalyubia, Sharkia and Minufiya governorates during 2012 and 2013. The multi-residue extraction, QuEChERS (quick natural cheap effective rugged and safe) was used for the pesticide residues. Gas chromatography coupled with FPD detector (GC- FPD) and high-performance liquid chromatography with a photodiode array detector (HPLC -PDA) was used for the determination of pesticides residues. Results revealed that all detected pesticide residues in fruit or leaves samples were above the accepted maximum residue limit (MRL). Oxamyl and profenofos were the most detected in fruit samples, 22.92% followed by carbofuran and triazophos (20.83% of samples each). In leaves, oxamyl was the most detected in 47.92% of samples followed by chlorpyrifos in 45.83% of samples. An increase in pesticides contaminated samples analysed in 2013 than in 2012 was observed. Furthermore, the samples of Qalyubia were the highest contaminated followed by the samples of Sharkia then Minufiya during the two investigated years.

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Keywords: Grape fruits; multi-residue extraction; gas chromatography; high-performance liquid chromatography; maximum residue limit (MRL).

1. INTRODUCTION

Vegetables and fruits are commonly used everywhere to meet the requirement of a balanced diet and good health [1]. Grape is one of the most widely-cultivated fruit crops in Egypt. It's regarded to be the second excellent fruit crop after citrus. Egyptian's geographical spread of production caused fresh sweet grape to be attainable from May to July. It's cultivated from Alexandria (in the north of Egypt) to Aswan (in the south). It was found several varieties of grape produced in Egypt, such as early sweet, Crimson, Superior, Thompson, Flame Seedless and Red Globe. Competition among Egyptian farmers is hard. There's always more competition each year because of the new grape cultivations coming into production. Thus the only thing that keeps one ahead of others in the market is the ability to produce high-quality grape through following Good Agriculture Practices (GAP) [2]. Grape leaves are widely consumed in traditional foods in many countries. They have positive health effects that used for diarrhoea, vomiting, varicose treatment and pharmaceutical preparations are patented and commercialised as drugs [3,4].

Pesticides are playing a significant role to maintain high agricultural productivity. Pesticides are used intensely, and many different types of pesticides applied extensively to fruits and vegetables, leading to their contamination by pesticide residues [5-11].

Pesticides contamination is a worldwide public health concern and a main international trade problem [12]. Pesticides are noxious substances and can persist in the environment for a long time. Pesticides have a risk to human health when they are applied indiscriminately [13]. Therefore, health points of view it is necessary to control the application of pesticides on crops [14,15]. Levels of pesticides should be monitored at an optimum position due to their relative toxicity to the environment and human health [16]. Thus, maximum residue levels (MRLs) for pesticides have established worldwide, which usually guide to manage the quantity of pesticides in foodstuffs.

The governmental monitoring programs for pesticide residues are essential means to respect for regulations and to design a database to evaluate the considerable number of pesticide residues and the concentration of residues intake. This data help notably to know human exposure to pesticide residues during dietary intake and also serve in improving pesticide action plans for the land. The monitoring program for pesticide residues should be established routinely to provide a check on compliance with best agricultural practice on the use of pesticides [17-20].

Optimally, pesticide monitoring should scan all pesticides in all samples. This requirement implies that, theoretically, all pesticides have to be detected in all items. In reality, because of what is known about the conventional pesticides used against the pests attacking grapes fruits and leaves, analyses have to be limited to those pesticides that are practically used, *i.e.* organophosphate and carbamate insecticides [21].

The aim of the present study was the monitoring of pesticide residues of organophosphates and carbamates in grape fruits and leaves in three Egyptian governorates, Qalyubia, Sharkia and Minufiya to compare these residues with the maximum residues limits in grapes.

2. MATERIALS AND METHODS

2.1 Sampling

A total of 24 samples of grape fruits and 24 samples of leaves were collected for multi-residue analyses of certain organophosphorus and carbamate pesticides. Samples were collected weekly from the local markets of three Governorates, Qalyubia, Minufiya and Sharkia (8 samples from each Governorate) during the period extending from 1st of July to the end of August of 2012, the sampling was repeated during the same period of the year 2013. Representative samples of fruits and leaves were prepared for residue analyses according to the guidelines of the Codex [22]. The subsamples were homogenized and kept at -20°C in a deep freezer until analysis (residues determination). The multiple residue analysis method of Lehotay et al. [23] to detect several pesticides in one analytical run was followed.

2.2 Extraction and Clean Up

Substances were extracted from grape fruits and leaves and purified according to the procedure described and modified by Lehotay et al. [23]. After chopped and homogenization of a 1 kg sample at high speed for 5 min, 10 g of the homogenized sample were weighed in a 50 ml centrifuge tube. Fifteen milliliters of 1% acidified acetonitrile with acetic acid were added. The screw cap was closed and vigorously shaken for 1 min using a Vortex mixer at maximum speed. Afterwards, 4 g of anhydrous $MgSO_4$, 1 g of NaCl and 1 g sodium citrate dehydrate were added, then extracted by shaking vigorously on Vortex for 2 min and centrifuged for 10 min at 5,000 rpm. An aliquot of 3 ml was transferred from the supernatant to new clean 15 ml centrifuge tube and cleaned up by dispersive 1 g of $MgSO_4$ with 75 mg of primary secondary amine (PSA), moreover it was added 0.02 g graphite carbon in case of grape leaves. Afterwards, centrifugation was carried out at 6,000 rpm for 5 min. An aliquot 2 ml from the supernatant was filtered through a 0.2 µm PTFE filter.

2.3 Pesticides Determination

Residues were determined using GC and HPLC. Working condition of the analyzed pesticides is presented in Tables 1, 2 and 3. Suitable aliquots (1 µl for GC & 20 µl for HPLC) from cleaned up extracted samples and standard pesticides solutions were injected.

Table 1. Temperature program of column oven

Level	Rate (°C/ min)	Temp (°C)	Time (min)
1	-	160	2
2	5	210	3
3	5	240	1
4	2	250	8

Total run time: 35 min

2.3.1 Organophosphorus

Gas chromatography (GC) Hewlett Packard (HP) serial 6890 equipped with flame phosphorus detector (FPD) to detect organophosphorus pesticides (525 nm filter). The gas chromatography instrument was adjusted for:

Injector temperature = 250°C
Detector temperature = 250°C
Flow rate of hydrogen =75 ml/min
Flow rate of air =100 ml/min
Flow rate of nitrogen =11.7 ml/min

Table 2. Columns used for organophosphorus pesticides

Column properties	Column A	Column B
Name	PAS-5	DB-1701
Film thickness	0.52 um	0.25um
Length	25 m	25 m
Column ID	0.32 mm	0.32 mm
Determined pesticides	OP[1]	OP[1] or OC[2] + PY[3]

1: Organophosphorus; 2: Organochlorine 3: Pyrethroids

2.3.2 Carbamates

Agilent 1260 HPLC system (USA) was used, with quaternary pump, auto sampler injector, thermostat compartment for the column and photodiode array detector. The chromatographic column was Zorbax C18 XDB 4.6 x 250 mm, 5 µm particle size, Agilent to HPLC, determination using gradient mobile solvent as shown in Table 3.

Table 3. Program of HPLC gradient mobile phase at flow rate (1.0 ml/min)

Step	Time (min)	Interval	% Water	% Acetonitril
Equil			82	18
0	0	0	82	18
1	0 - 1	1	60	40
2	1 - 3	2	20	80
3	3 - 6	3	10	90
4	6 - 10	4 - 7	0	100

2.4 Pesticides Monitored

Twenty - six insecticides were studied for the identification and quantification, which are mainly of two groups, organophosphorus (21) and carbamates (5). Their common names, limits of determination, spiking levels, average recoveries and retention times are illustrated and presented in Table 4.

3. RESULTS AND DISCUSSION

3.1 Pesticide Residues in Grape Fruits

Data revealed generally that oxamyl and profenofos were the most detected in fruit

samples of grape during the two investigated years. Oxamyl followed by chlorpyrifos were the most in samples of leaves. Furthermore, fruits and leaves samples were contaminated by the pesticide residues in 2013 more than in 2012. On the other hand, although chlorpyrifos-methyl was not detected in fruit samples of 2012, it was found in samples of 2013. As well as pirimiphos-methyl, cadusafos and fenitrothion were detected only in leaves samples of 2013 (Table 5).

The mean values of pesticide residues determined in all fruits and leaves samples were higher than MRLs. This fact was clearly observed when these residues were compared with MRL established by European Union [24]. Results also demonstrated that Qalyubia governorate had the highest contaminated fruit samples followed by Sharkia then Minufiya in the two investigated years.

In 2012, profenofos, triazophos and azinophos-ethyl were found in samples of the three studied governorates. Profenofos contaminated 37.5, 12.5 and 25% of Qalyubia, Sharkia and Minufiya samples at mean values of 0.199, 0.017 and 0.058 mg kg^{-1}, respectively. The mean values of triazophos were 0.088, 0.040 and 0.021 mg kg^{-1} in 25% of samples of each Qalyubia and Sharkia and in 12.5% of Minufiya samples, respectively. Residues of Azinophos-ethyl were detected in 12.5% of samples collected from Qalyubia and Minufiya each and in 25% of Sharkia samples, respectively, at mean values of 0.457, 0.069 and 0.436 mg kg^{-1}, respectively.

Diazinon was detected in samples of only two governorates, Qalyubia (25%) and Sharkia (12.5%) at mean values of 0.051 and 0.039 mg kg^{-1}, respectively. Residues of pyrazophos were found only in samples of both Sharkia (37.5%) and Minufiya (25%) at mean values of 0.078 and 0.070 mg kg^{-1}, respectively. Prothifos and chloropyrifos contaminated samples of only one governorate, Qalyubia at mean values 0.030 and 0.151 mg kg^{-1} for 12.5% and 25% of its samples, respectively.

Regarding carbamate pesticide residues, 25% of samples of Qalyubia were contaminated by carbofuran. Each of oxamyl, methomyl and carbaryl were detected in 12.5% of samples at mean values of 0.313, 0.428, 1.32 and 0.921 mg kg^{-1}, respectively. In samples of Sharkia, only methomyl was not found, 25% of samples were contaminated by oxamyl at a mean value of 0.467 mg kg^{-1}, while each of carbofuran and carbaryl contaminated 12.5% of samples at mean values of 0.240 and 0.710 mg kg^{-1}, respectively. In Minufiya 25% of its samples were contaminated by oxamyl at a mean value of 0.410 mg kg^{-1}, while each of the methomyl, carbofuran and carbaryl contaminated 12.5% of samples at mean values of 0.800, 0.933 and 0.828 mg kg^{-1}, respectively (Table 6).

In 2013, profenofos, diazinon and triazophos were found in samples of the three investigated governorates. Profenofos was detected in 37.5% of fruit samples of Qalyubia and 12.5% of Sharkia and Minufiya samples each at mean values of 0.384, 0.082 and 0.178 mg kg^{-1}, respectively. Diazinon residues contaminated 25% of Qalyubia samples at a mean value of 0.134 mg kg^{-1}, and 12.5% of Sharkia and Minufiya samples each at mean values of 0.726 and 0.617 mg kg^{-1}, respectively. Mean values of triazophos were 0.384, 0.060 and 0.118 mg kg^{-1} in 25% of samples of each Qalyubia, Sharkia and 12.5% of samples from Minufiya, respectively. Chlorpyrifos-methyl residues were detected only in samples of both Qalyubia (37.5%) and Minufiya (12.5%) at mean values of 2.223 and 1.130 mg kg^{-1}, respectively. Chlorpyrifos residues were found in 25% of Qalyubia and Sharkia samples each at mean values of 2.562 and 1.192 mg kg^{-1}, respectively. Prothifos contaminated only Qalyubia samples at mean values of 0.062 mg kg^{-1} for 12.5% of its samples.

Oxamyl, methomyl, carbofuran and carbaryl were detected in 25, 12.5, 25 and 12.5% of Qalyubia samples at mean values of 0.602, 0.991, 0.255 and 0.226 mg kg^{-1}, respectively. In samples of Sharkia, only methomyl was not found. Oxamyl, carbofuran and carbaryl contaminated the samples at mean values of 1.775, 0.586 and 0.330 mg kg^{-1} for 25, 25 and 12.5% of samples, respectively. In Minufiy samples, oxamyl, methomyl, carbofuran and carbaryl were found in 25, 37.5, 25 and 12.5% of samples at mean values of 0.678, 1.244, 1.292 and 0.436 mg kg^{-1}, respectively (Table 7).

3.2 The Pesticide Residues in Grape Leaves

The results indicated that in year 2012, profenofos, chlorpyrifos, triazophos and azinphos-ethyl contaminated samples collected from the three governorates. Profenofos was detected in 50% of Sharkia samples at a mean value of 1.398 mg kg^{-1}, while 37.5% of Qalyubia and Minufiya samples each contaminated at

mean values of 1.812 and 1.575 mg kg^{-1}, respectively. Chlorpyrifos residues were detected in 62.5% of Minufiya samples at a mean value of 1.704 mg kg^{-1}, it was also found in 37.5% of Qalyubia and Sharkia samples each at mean values of 1.710 and 1.150 mg kg^{-1}, respectively. Triazophos contaminated 25% of Qalyubia, Sharkia and Minufiya samples each at mean values of 0.791, 1.120 and 0.402 mg kg^{-1}, respectively. Azinphos-ethyl residues were detected in 25% of Qalyubia and Sharkia samples each at mean values of 1.429, 0.566 mg kg^{-1}, respectively, while it was found in 12.5% of Minufiya samples at a mean value of 0.418 mg kg^{-1}. Diazinon was found in 50 and 37.5% of only samples of Qalyubia and Sharkia at mean values of 1.877 and 1.269 mg kg^{-1}, respectively.

Pyrazophos and chlorpyrifos methyl residues were detected only in samples collected from both Sharkia and Minufiya. In Sharkia they contaminated 37.5% of samples at mean values of 0.674 and1.738 mg kg^{-1}, respectively whereas in Minufiya they were found in 25 and 50% of samples at mean values of 0.661 and 2.099 mg kg^{-1}, respectively. Only samples of Qalyubia were contaminated by prothifos, phenthoate and cyanophos each at mean values of 0.095, 3.059 and 1.527 mg kg^{-1} for 37.5, 25 and 12.5% of their samples, respectively.

Table 4. The limits of determination (LOD), spiking levels, average recoveries and retention times of the investigated organophosphorus and carbamate compounds

Pesticides	LOD (mg kg^{-1})	Spiking levels (mg kg^{-1})			Average recovery %	Retention Time (min)	
Organophosphorus						**Colum A**	**Colum B**
Dichlorvos	0.001	0.5	0.25	0.001	94.33	1.491	3.542
Methamidophos	0.001	0.5	0.25	0.001	80.92	1.713	3.914
Ethoprophos	0.001	0.5	0.25	0.001	92.41	3.922	4.932
Cadusafos	0.001	0.5	0.25	0.001	88.53	4.497	5.894
Phorate	0.001	0.5	0.25	0.001	83.75	4.879	6.283
Diazinon	0.001	0.5	0.25	0.001	98.26	5.799	7.438
Disulfoton	0.005	0.5	0.25	0.005	95.25	6.090	8.176
Cyanophos	0.005	0.5	0.25	0.005	82.77	7.138	9.247
Chlorpyrifos-methyl	0.001	0.5	0.25	0.001	87.96	7.398	10.041
Pirimiphos-methyl	0.002	0.5	0.25	0.002	92.21	8.073	10.868
Chlorpyrifos	0.001	0.5	0.25	0.001	95.32	8.471	11.299
Fenitrothion	0.010	0.5	0.25	0.010	86.55	8.607	11.773
Phenthoate	0.001	0.5	0.25	0.001	86.92	9.392	12.192
Prothiofos	0.002	0.5	0.25	0.002	91.75	10.221	13.343
Profenofos	0.002	0.5	0.25	0.002	93.55	11.631	14.578
Fenamiphos	0.010	0.5	0.25	0.010	84.34	11.822	15.233
Ethion	0.005	0.5	0.25	0.005	83.89	13.430	16.742
Triazophos	0.001	0.5	0.25	0.001	95.63	14.094	17.027
Azinphos-methyl	0.001	0.5	0.25	0.001	81.29	17.725	21.201
Pyrazophos	0.010	0.5	0.25	0.010	79.94	18.728	22.492
Azinphos-ethyl	0.001	0.5	0.25	0.001	84.25	18.839	23.017
Carbamates							
Oxamyl	0.010	0.5	0.25	0.010	95.69	2.408	
Methomyl	0.010	0.5	0.25	0.010	96.79	2.730	
Aldicarb	0.100	0.5	0.25	0.100	89.96	4.356	
Carbofuran	0.010	0.5	0.25	0.010	92.63	5.573	
Carbaryl	0.010	0.5	0.25	0.010	94.22	6.046	

Column A: PAS-5; Column B: DB-1701

Table 5. Contaminated samples of grape fruits and leaves with OP and Carb compounds collected from the three investigated governorates in 2012 and 2013

Detected pesticides	Fruits		Leaves	
	No. of contaminated samples		No. of contaminated samples	
	2012	2013	2012	2013
Oxamyl	5	6	10	13
Profenofos	6	5	10	6
Carbofuran	4	6	8	8
Triazophos	5	5	6	2
Diazinon	3	4	7	13
Chlorpyrifos	2	4	11	11
Methomyl	2	4	8	11
Azinphos-ethyl	4	2	5	2
Carbaryl	3	3	3	2
Pyrazophos	5	ND	5	2
Chlorpyrifos-methyl	ND	4	7	12
Prothiofos	1	1	3	6
Phenthoate	ND	ND	2	2
Cyanophos	ND	ND	1	ND
Pirimiphos-methyl	ND	ND	ND	2
Cadusafos	ND	ND	ND	2
Fenitrothion	ND	ND	ND	3
Total	40	44	86	97

ND: Not detected

Oxamyl, methomyl, carbofuran and carbaryl were found in 50, 37.5, 50 and 12.5% of Qalyubia samples at mean values of 0.974, 1.174, 0.255 and 0.521 mg kg^{-1}, respectively. Also, oxamyl, methomyl, carbofuran and carbaryl were detected of Minufiya samples at mean values of 1.299, 0.816, 0.102 and 0.953 mg kg^{-1} in 37.5, 37.5, 12.5 and 12.5% of samples, respectively. In Sharkia, 37.5, 25, 37.5 and 12.5% of its samples were contaminated by oxamyl, methomyl, carbofuran and carbaryl at mean values of 0.679, 0.844, 0.810 and 0.416 mg kg^{-1}, respectively (Table 8).

In the year 2013, diazinon, profenofos, chlorpyrifos and chlorpyrifos-methyl were detected in samples collected from the three governorates. Diazinon was found in 62.5% of Qalyubia samples at a mean value of 2.174 mg kg^{-1}, it also contaminatedalso 50% of Sharkia and Minufiya samples each at mean values of 1.399 and 1.261 mg kg^{-1}, respectively. Profenofos was detected in 37.5, 25 and 12.5% of samples of Qalyubia, Sharkia and Minufiya at mean values of 1.864, 0.897 and 0.581 mg kg^{-1}, respectively. Chlorpyrifos residues were detected in 50% of Qalyubia and Sharkia samples each, and 37.5% of Minufiya samples at mean values of 1.039, 1.213 and 0.928 mg kg^{-1}, respectively. Chlorpyrifos-methyl residues contaminated samples of 37.5, 50 and 62.5% of Qalyubia, Sharkia and Minufiya at mean values of 3.622, 1.773 and 2.038 mg kg^{-1}, respectively.

Prothifos and triazophos were found only in samples of Qalyubia and Sharkia. Prothifos contaminated 50 and 25% of their samples at mean values of 0.203 and 0.053 mg kg^{-1}, respectively, while triazophos was detected at mean values of 0.516 and 0.291 mg kg^{-1} in 12.5% of Qalyubia and Sharkia samples, respectively. Azinphos-ethyl residues were found in 12.5% of Sharkia and Minufiya samples each at mean values of 0.301 and 0.237 mg kg^{-1}, respectively.

Phenthoate, cadusafos and pirimiphos-methyl were detected in 25% of only Qalyubia samples at mean values of 1.333, 1.168 and 1.124 mg kg^{-1}, respectively.

Pyrazophos and fenitrothion were found only in samples from Minufiya. Pyrazophos was detected in 25% of its samples at a mean value 0.324 mg kg^{-1}. Fenitrothion was detected in 37.5% of samples at a mean value of 1.031 mg kg^{-1}.

Table 6. Contaminated samples of grape fruits with OP and Carb compounds collected from the three investigated governorates in 2012

Governorate		Total no. of analyzed samples	Detected pesticides		No. of contaminated samples	Minimum	Maximum	Mean	MRLs
						(mg kg^{-1})			
Governorate	Qalyubia	8	OP	Diazinon	2	0.029	0.072	0.051	0.010
				Prothiofos	1	0.030	0.030	0.030	0.010
				Profenofos	3	0.015	0.329	0.199	0.010
				Triazophos	2	0.063	0.113	0.088	0.010
				Azinphos-ethyl	1	0.046	0.457	0.457	0.020
				Chlorpyrifos	2	0.068	0.233	0.151	0.010
			Carb	Oxamyl	1	0.428	0.428	0.428	0.010
				Methomyl	1	1.320	1.320	1.320	0.300
				Carbofuran	2	0.062	0.563	0.313	0.002
				Carbaryl	1	0.921	0.921	0.921	0.010
	Sharkia	8	OP	Diazinon	1	0.039	0.039	0.039	0.010
				Profenofos	1	0.017	0.017	0.017	0.010
				Triazophos	2	0.024	0.055	0.040	0.010
				Azinphos-ethyl	2	0.062	0.810	0.436	0.020
				Pyrazophos	3	0.014	0.140	0.078	0.010
			Carb	Oxamyl	2	0.109	0.825	0.467	0.010
				Carbofuran	1	0.240	0.240	0.240	0.002
				Carbaryl	1	0.710	0.710	0.710	0.010
	Minufiya	8	OP	Profenofos	2	0.017	0.100	0.058	0.050
				Triazophos	1	0.021	0.021	0.021	0.010
				Azinphos-ethyl	1	0.069	0.069	0.069	0.020
				Pyrazophos	2	0.046	0.095	0.070	0.050
			Carb	Oxamyl	2	0.352	0.468	0.410	0.010
				Methomyl	1	0.800	0.800	0.800	0.300
				Carbofuran	1	0.933	0.933	0.933	0.002
				Carbaryl	1	0.828	0.828	0.828	0.010

Oxamyl, methomyl, carbofuran and carbaryl contaminated 62.5, 50, 37.5 and 12.5% of Qalyubia samples at mean values of 1.215, 1.245, 0.749 and 0.107 mg kg^{-1}, respectively. In Sharkia samples, oxamyl, methomyl and carbofuran were detected at mean values of 1.067, 1.601 and 0.435 mg kg^{-1} in 50, 37.5 and 37.5% of its samples, respectively. Oxamyl, methomyl, carbofuran and carbaryl were detected of Minufiya samples at mean values of 1.126, 1.565, 0.160 and 0.101 mg kg^{-1} in 50, 50, 25 and 12.5% of its samples, respectively (Table 9).

The results of this study are consistent with the study carried out by Dogheim et al. [7], who determined multi-residues of pesticides in 1579 samples of Egyptian fruits and vegetables collected from 8 local markets in 6 governorates during 1996 for 53 pesticide residues including organophosphorus and organonitrogen compounds as well as some synthetic pyrethroids. They found that 23.9% of samples contained detectable residues, for individual crops, contaminated samples ranged from 0 to 96% of the number of samples analyzed.

The occurrence of multi-residue pesticide contamination in grapes has also been reported in other investigations [25], high level of pesticide residues was found in grapes in Lithuania in the Brazilian pesticide residues monitoring program. The most frequently detected pesticide residues were chlorpyrifos, methidathion, imazalil, thiabendazole, maneb group, procymidone, lambda-cyhalothrin, carbendazim, iprodione, endosulfan, orthophenylphenol, vinclozolin, pyrimethanil, fenhexamid, prochloraz, cyprodinil and boscalid. As well as, Reddy et al. [26] reported that the insecticide residues in market samples of grapes were chlorpyriphos, monocrotophos, acephate, methamidophos and quinalphos.

Furthermore, the results obtained however did not correspond with the results obtained by

Mutengwe et al. [27], who reported that in fruits and vegetables sold at two of the biggest fresh produce markets in Africa, a total of 199 fruit and vegetable samples were collected between 2012 and 2014 and analyzed for 74 pesticides commonly used in the horticultural sector. Of the samples analyzed, 91% were compliant with set maximum residue levels (MRLs). This might be attributed to these pesticides being misused, overused or unnecessarily used by farmers who have very limited or no information about how to apply pesticides or their health implications when present in foodstuffs and are just after a harvest.

Also, it was found that among the 300 fruit and vegetable samples screened between December 1990 and September 1992 in Pakistan, 121 samples contained a variety of pesticides mainly insecticides. Thirty-eight samples contained pesticide residues above the MRLs proposed by FAO/WHO [8]. Data of Allen et al. [28] and Osman et al. [29] showed also that a multi-residue pesticide contamination was found in various commodities [28,29]. Mutengwe et al. [27] found that samples containing more than one pesticide residue constituted 4.02% of all samples tested. Iprodione and imazalil were detected to be the most frequently determined pesticides (12 samples each). Profenofos, boscalid, procymidone and endosulfan were associated with the most noncompliance, including exceeding MRL values or being unregistered for the specific crop.

Table 7. Contaminated samples of grape fruits with OP and Carb compounds collected from the three investigated governorates in 2013

		Total no. of analyzed samples		Detected pesticides	No. of contaminated samples	Minimum	Maximum	Mean	MRLs
						(mg kg^{-1})			
Governorate	Qalyubia	8	OP	Diazinon	2	0.068	0.199	0.134	0.010
				Prothiofos	1	0.062	0.062	0.062	0.010
				Profenofos	3	0.043	0.855	0.385	0.010
				Triazophos	2	0.044	0.723	0.384	0.010
				Chlorpyrifos-methyl	3	1.208	3.210	2.223	0.020
				Chlorpyrifos	2	0.234	4.890	2.562	0.010
			Carb	Oxamyl	2	0.516	0.687	0.602	0.010
				Methomyl	1	0.991	0.991	0.991	0.300
				Carbofuran	2	0.181	0.328	0.255	0.002
				Carbaryl	1	0.226	0.226	0.226	0.010
	Sharkia	8	OP	Diazinon	1	0.726	0.726	0.726	0.010
				Profenofos	1	0.082	0.082	0.082	0.010
				Triazophos	2	0.033	0.087	0.060	0.010
				Azinphos-ethyl	2	0.066	0.920	0.493	0.020
				Chlorpyrifos	2	0.133	2.250	1.192	0.010
			Carb	Oxamyl	2	1.210	2.340	1.775	0.010
				Carbofuran	2	0.361	0.811	0.586	0.002
				Carbaryl	1	0.330	0.330	0.330	0.010
	Minufiya	8	OP	Diazinon	1	0.617	0.617	0.617	0.010
				Profenofos	1	0.178	0.178	0.178	0.010
				Chlorpyrifos-methyl	1	1.130	1.130	1.130	0.020
				Triazophos	1	0.118	0.118	0.118	0.010
			Carb	Oxamyl	2	0.441	0.914	0.678	0.010
				Methomyl	3	0.521	1.990	1.244	0.300
				Carbofuran	2	0.754	1.830	1.292	0.002
				Carbaryl	1	0.174	0.697	0.436	0.010

Table 8. Contaminated samples of grape leaves with OP and Carb compounds collected from the three investigated governorates in 2012

Governorate		Total no. of analyzed samples	Detected pesticides		No. of contaminated samples	Minimum	Maximum	Mean	MRLs
						(mg kg^{-1})			
Governorate	Qalyubia	8	OP	Diazinon	4	0.918	3.110	1.877	0.010
				Prothiofos	3	0.061	0.135	0.095	0.010
				Profenofos	3	1.321	2.430	1.812	0.010
				Triazophos	2	0.421	1.160	0.791	0.010
				Azinphos-ethyl	2	0.867	1.990	1.429	0.020
				Chlorpyrifos	3	0.519	2.951	1.710	0.010
				Phenthoate	2	2.238	3.880	3.059	0.020
				Cyanophos	1	1.527	1.527	1.527	0.200
			Carb	Oxamyl	4	0.211	1.678	0.974	0.010
				Methomyl	3	0.589	1.542	1.174	0.300
				Carbofuran	4	0.142	1.468	0.255	0.002
				Carbaryl	1	0.521	0.521	0.521	0.010
	Sharkia	8	OP	Diazinon	3	0.651	2.031	1.269	0.010
				Profenofos	4	0.422	2.337	1.398	0.010
				Triazophos	2	0.771	1.468	1.120	0.010
				Azinphos-ethyl	2	0.398	0.734	0.566	0.020
				Pyrazophos	3	0.094	1.110	0.674	0.010
				Chlorpyrifos	3	0.549	2.591	1.150	0.010
				Chlorpyrifos-methyl	3	0.899	2.584	1.738	0.020
			Carb	Oxamyl	3	0.092	1.449	0.679	0.010
				Methomyl	2	0.692	0.995	0.844	0.300
				Carbofuran	3	0.387	1.081	0.810	0.002
				Carbaryl	1	0.416	0.416	0.416	0.010
	Minufiya	8	OP	Profenofos	3	0.866	1.369	1.575	0.050
				Triazophos	2	0.134	0.669	0.402	0.010
				Azinphos-ethyl	1	0.418	0.418	0.418	0.020
				Pyrazophos	2	0.358	0.964	0.661	0.050
				Chlorpyrifos	5	0.383	3.446	1.704	0.010
				Chlorpyrifos-methyl	4	0.998	2.916	2.099	0.020
			Carb	Oxamyl	3	0.891	1.658	1.299	0.010
				Methomyl	3	0.367	1.215	0.816	0.300
				Carbofuran	1	0.102	0.102	0.102	0.002
				Carbaryl	1	0.953	0.953	0.953	0.010

Moreover, pesticide residues of diazinon and oxamyl were detected in many analyzed samples that are classified as highly hazardous [30]. The present data also agree with the previous study of Torres et al. [9], who analyzed 200 samples of lemons, grape fruits and oranges in Spain, to establish the residue levels of 12 organophosohorus, in the period 1994-1995, some of which were chlorpyrifos, diazinon, fenitrothion, malathion and parathion-methyl. A total of 32.25% of the samples contained pesticide residues and 6.9% of them exceeded the European Union MRLs, parathion-methyl residues were above MRLs. In the present study, the occurrence of multiple residues in the most analyzed samples might be attributed to pesticides misused, overused or a consequence of the application of various types of pesticides to protect the grape crops against different insect pests. Pesticide application frequency in vegetable crops ranged from twice a month to once a week depending on the crop [19]. This problem is further exacerbated by farmers' limited knowledge of pesticide safety. They were not following proper precautions with regard to the application of pesticides in appropriate

dosages and at standard pre-harvest intervals. Hence, this could lead to health risks not only to the farmers but also to the general consumers [20]. As a frequent observation, most of the detected pesticides were not recommended for the use on grapes according to the recommendations of the Egyptian Ministry of Agriculture 2017. This observation reflects the fact that the usage of pesticides in Egypt for agricultural activities is out of control. Therefore, remarkably high incidence rates of pesticides detected give warning to the Egyptian government to perform proper regulations on pesticide monitoring program. To minimize the impact of pesticide residues, it must be used the preventive measures such as rational use of pesticides, use of natural pesticides and bio-pesticides, practicing organic farming, washing and proper processing of food products and strict implementation of pesticide-related laws [31].

Table 9. Contaminated samples of grape leaves with OP and Carb compounds collected from the three investigated governorates in 2013

		Total no. of analyzed samples	Detected pesticides		No. of contaminated samples	Minimum	Maximum	Mean	MRLs
						(mg kg^{-1})			
Governorate	Qalyubia	8	OP	Diazinon	5	0.888	3.157	2.174	0.010
				Prothiofos	4	0.075	0.308	0.203	0.010
				Profenofos	3	1.219	2.273	1.864	0.010
				Triazophos	1	0.516	0.516	0.516	0.010
				Pirimiphos-methyl	2	0.926	1.321	1.124	0.010
				Chlorpyrifos	4	0.316	1.965	1.039	0.010
				Phenthoate	2	0.995	1.671	1.333	0.020
				Cadusafos	2	0.354	1.982	1.168	0.010
				Chlorpyrifos-methyl	3	2.361	4.895	3.622	0.020
			Carb	Oxamyl	5	0.149	2.491	1.215	0.010
				Methomyl	4	0.511	2.113	1.245	0.300
				Carbofuran	3	0.098	1.328	0.749	0.002
				Carbaryl	1	0.107	0.107	0.107	0.010
	Sharkia	8	OP	Diazinon	4	0.398	2.966	1.399	0.010
				Profenofos	2	0.866	0.927	0.897	0.010
				Triazophos	1	0.291	0.291	0.291	0.010
				Azinphos-ethyl	1	0.301	0.301	0.301	0.020
				Prothiofos	2	0.020	0.087	0.053	0.010
				Chlorpyrifos	4	0.149	2.146	1.213	0.010
				Chlorpyrifos-methyl	4	0.738	2.951	1.773	0.020
			Carb	Oxamyl	4	0.234	1.972	1.067	0.010
				Methomyl	3	0.814	2.136	1.601	0.300
				Carbofuran	3	0.119	0.819	0.435	0.002
	Minufiya	8	OP	Diazinon	4	0.346	2.517	1.261	0.050
				Profenofos	1	0.581	0.581	0.581	0.010
				Azinphos-ethyl	1	0.237	0.237	0.237	0.020
				Pyrazophos	2	0.161	0.486	0.324	0.050
				Chlorpyrifos	3	0.446	1.358	0.928	0.010
				Chlorpyrifos-methyl	5	0.917	3.101	2.038	0.020
				Fenitrothion	3	0.398	1.485	1.031	0.010
			Carb	Oxamyl	4	0.533	1.658	1.126	0.010
				Methomyl	4	0.739	2.016	1.565	0.300
				Carbofuran	2	0.054	0.266	0.160	0.002
				Carbaryl	1	0.101	0.101	0.101	0.010

4. CONCLUSION

The results indicated that grapefruits and leaves samples were contaminated with pesticide residues at concentrations above the MRL. Hence, there is a need for continuous survey and monitoring programs for pesticide residues in food commodities to protect the end-user from indiscriminate exposure to pesticides. It is recommended to conduct educational programs for farmers on the control and safer use of pesticides. Regulatory policies on pesticides should also be done to protect farmers' and consumers' health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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