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Monitoring of Pesticide Residues in Cucumber Samples Marketed in Egypt

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ABSTRACT

Pesticide residues in vegetables are considered a potential risk to consumers and a human health concern. Herein, we performed determination and quantification of pesticides, metabolites, and isomers residues by LC-MS/MS and GC-MS in cucumber samples collected from six markets in two governorates in Egypt, i.e. Giza (Dokki) and Assiut (El-Zahraa, El-Gomhoria, El-Welidia, El-Fath, and Abnoub). As a result, a total of 12 pesticide residues were found. One of them slightly exceeded the maximum residue levels (MRLs) (Thiophanate-methyl= 0.12 mg/kg). Iprodione and propamocarb were the most frequently found pesticides. Furthermore, there were no health risk index recorded among the pesticide residues. A regular pesticide residues analysis program should be applied to monitoring and determine the pesticide residues in vegetables to keep the food safe in Egypt.

Keywords: Pesticide residues, maximum residue levels (MRLs), cucumber, LC-MS/MS, QuEChERS, monitoring

INTRODUCTION

In Egypt, chemical pesticides are commonly applied in agricultural sector to improve productivity and pest control. However, pesticide residues can constitute a potential risk to consumers which considered a human health concern (Ahmed *et al*., 2014a; Ahmed *et al*., 2016; Ibrahim *et al*., 2018). In this regards, analysis of pesticide residues in food is an essential requirement for numerous aspects such as consumers, producers, and food quality control authorities (Shams El Din *et al*., 2012; Ahmed *et al*., 2014b). Thus, it is very remarkable to facilitate various multi-residue methods for detecting pesticide residues in vegetables samples which preclude the possible health risks (Ramadan *et al*., 2016; Zou *et al*., 2017). Therefore, the present study focused to provide strong pattern of information on the contamination levels of pesticide residues in cucumber samples collected from six markets in two governorates in Egypt, i.e. Giza (Dokki) and Assiut (El-Zahraa, El-Gomhoria, El-Welidia, El-Fath, and Abnoub) using LC-MS/MS and GC-MS.

MATERIALS AND METHODS

Sample collection

Six samples (2 kg each) were collected from six local markets in 2 major cities in Egypt in July 2018. The gathered samples were wrapped and placed immediately in an ice container at 4 °C, and tagged by the name of the market and city and then sent to the laboratory for analysis. Upon arrival of samples at the laboratory, they were inspected and checked. Samples numbers were assigned and stored frozen until analysis (Table 1).

Table 1. Random markets from which the cucumber samples obtained in Egypt

Market	City
Dokki	Giza
El-Zahraa	Assiut
El-Gomhoria	Assiut
El-Welidia	Assiut
El-Fath	Assiut
Abnoub	Assiut

Sample preparation

Sample preparation were previously described by Ahmed *et al*, 2016. Briefly, samples were properly homogenized in blender and a portion of which is taken for analysis. Water, acetonitrile and if necessary formic acid was added to the milled sample. Samples were extracted with Acetonitrile and QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction salts. The sample was shaken and further cleaned up dSPE considered to improve better sensitivity. After shaking and another centrifugation the extracts were ready for analysis by LC-MS/MS and GC-MS.

Preparation of pesticide standards

Stock standards were received in ampoules and stored in appropriate glass container. Primary stock solutions were prepared from the pure reference materials. Working solutions were prepared from the stock solutions or other working solutions. 100 µl of stock standard solution each were taken and diluted to 1 ml. The working standard solutions were prepared by pipetting an accurate amount of the stock solution into an appropriate volumetric flask and then diluted (Table 2, 3).

Table 2. Preparation of pesticide standards (Stock and working St. Solutions).

Stock standard concentration (mg/L)	Volume of stock standard(ml)	Final volume (mL)	Final concentration (mg/L)	Label
100	0.1	1	10	Stock St.
10	0.1	1	1	WS 1
1	0.1	1	0.1	WS 2

WS: Working Standard

Table 3. Preparation of calibration curve standard

Standard concentration (mg/L)	Volume of working st. [Aliquot volume (ml)]	Final volume (mL)	Final concentration (mg/L)	Solvent for dilution
0.1	0.05	1	0.005	(Methanol + water) (1:1)
0.1	0.1	1	0.01	
0.1	0.2	1	0.02	
0.1	0.4	1	0.04	
1	0.1	1	0.1	

Instrument conditions

The calculations based mainly on the LC-MS/MS results (Table 4). Peak area was used to calculate the standard curve. The concentration of the unknown was calculated from the equation using regression analysis of

the reciprocal of the analyte concentration as weighting factor (1/x) by using the equation below:

$$Y = mX + C \quad (1)$$

Where, Y = Analyte area, X = concentration of analyte, m = slope of the calibration curve, and C = Y-axis intercept value. Final pesticide residue present in the sample is in µg/Kg or mg/kg

Table 4. LC-MS/MS instrument conditions

Instrument:	LC-MS/MS Tandam mass Spectrometer, AB SCIEX Model: QTRAP 4500	
Column:	Phenomenexsynergi Fusion RP-C18,2.5 µm, 50 X 2 mm	
Mobile phase:	A: Water with 5% Ammonium formate/ acetate B: Methanol with 5% Ammonium formate/ acetae	
Flow rate	0.4 ml/min.	
Injection Volume	5µl	
Tray Temp	10 °C	
Gradient Program for Positive Mode		
Time (min.)	A%	B%
1	95	5
10	50	50
10	5	95
18	95	5

GC-MS system – Agilent 7890B

The GC- MS system was used to scan and analyze the samples. After the instrument has been tuned and calibrated, the GC and MS methods created and the sample list built started the sample analysis (Table 5).

Table 5. GC- MS instrument conditions:

Injector temp.	250^{O}C
Carrier Gas	Helium 1 ml/ min.
MS Scan time	0.2 sec.
MS ion source temp.	220^{O}C
MS scan range	m/z 40 - 500
MS inter scan delay	0.01

Method validation

Results were reported as ppm (mg/kg) in this study (Tables 6A and 6B). Replicate measurements of lowest concentrations spiked test samples at least 5 times. Lowest spike level (0.01 PPM (0.01 mg/kg) meeting the method performance criteria for trueness (mean recoveries are within the range 76–106% (acceptable range 70 – 120%) and precision (repeatability RSD ≤ 20 %). LOD ranged from 0.0017 to 0.0046 mg/kg and LOQ ranged from 0.0056 to 0.0155 mg/ kg. It has been noted that LOQ < MRL. While at spike level 0.1 ppm the results were more precise where mean recoveries are within the range 80 – 108% and precision (repeatability RSD ≤ 16.6 %).

Table 6 A. The average recovery percentage (spike level 0.01 ppm) and other validated parameters of analytes in cucumber samples.

Analyte	Average determined value for 5 replicates (mg/kg)	Average recovery %	SD	RSD %*	LOD = 3*SD mg/ kg	LOQ = 10*SD mg/ kg
Abamectin	0.0103	89.6	0.000847	9.45	0.0025	0.0085
Carbendazim	0.0093	91.6	0.00155	16.92	0.0046	0.0155
Thiophanate-methyl	0.0104	101.2	0.00149	14.68	0.0045	0.0149
Metalaxyl	0.0105	98.6	0.00103	10.46	0.0031	0.0103
Imidacloprid	0.0093	93.8	0.00127	13.54	0.0038	0.0127
Chlorpyrifos	0.0075	88.3	0.000563	6.78	0.0017	0.0056
Iprodione	0.0079	88.8	0.000988	11.13	0.0030	0.0099
Propamocarb	0.011	87.4	0.00137	15.68	0.0041	0.0137
Buprofezin	0.00108	75.8	0.00136	17.88	0.0041	0.0135
Fenpyroximate	0.0092	80.6	0.000669	8.3	0.0020	0.0067
Emamectin	0.0076	84	0.000834	9.93	0.0025	0.0083
Azoxystrobin	0.0106	105.8	0.00124	11.7	0.0037	0.0124

***RSD% = (SD/Mean) x 100**

Table 6 B. The average recovery percentage (spike level 0.1 ppm) and other validated parameters of analytes in cucumber samples.

Analyte	Average determined value for 5 replicates (mg/kg)	Average recovery %	SD	RSD %*
Abamectin	0.1034	103.4	0.010761	10.41
Carbendazim	0.097	97	0.01118	11.18
Thiophanate-methyl	0.0928	92.8	0.013461	14.51
Metalaxyl	0.082	82	0.005891	6.83
Imidacloprid	0.10782	107.82	0.011392	10.57
Chlorpyrifos	0.0874	87.4	0.013557	15.51
Iprodione	0.08326	83.3	0.006373	7.65
Propamocarb	0.10668	106.7	0.010838	10.16
Buprofezin	0.0882	88.2	0.013442	15.24
Fenpyroximate	0.0904	90.4	0.010854	12.01
Emamectin	0.0796	79.6	0.000834	10.48
Azoxystrobin	0.10706	105.8	0.009668	9.30

Linearity and range R^2 value

The linearity of an analytical procedure is its ability within a given range to obtain test results directly proportional to the concentration (amount) of analyte in the sample. Five levels concentrations spaced across the linear range. Plot response (y axis) against concentration (x axis). The lower end of the working range is bounded by the limit of quantification LOQ. The results showed Linearity R^2 value ranged from 0.992 to 0.999 (acceptable range > 0.99).

RESULTS AND DISCUSSION

Data in table 7 demonstrated the level of pesticide residues in cucumber samples. Twelve pesticides (6 fungicides and 6 insecticides and acaricides) residues were detected in the market samples. The most frequently pesticide residues were iprodione (El-Zahraa, El-Welidia, El-Fath, and Abnoub markets) and propamocarb (El-Zahraa, El-Gomhoria, El-Welidia, and Abnoub markets) followed by carbendazim (Dokki, El-Zahraa, El-Gomhoria markets), metalaxyl (El-Zahraa, El-Welidia, and El-Fath), and azoxystrobin (El-Gomhoria, El-Welidia, and El-Fath). However, thiophanate-methyl was considered the only pesticide residue that slightly exceeded the MRL level (0.12 mg/kg).

Importantly, it is substantial to evaluate the estimate daily intake (EDI) because it is the realistic estimate of pesticide residue that calculated with the perspective of international guidelines (FAO, 2009) which is expressed as microgram of pesticides per kilogram body weight per day (µg/ kg b.w./ day) and calculated from the following equation:

$$EDI = \Sigma\ C \times F / D \times W \qquad (2)$$

where C is the sum of the concentration of pesticide in each location (µg/kg), F is the mean annual intake of food per person, D is number of days in a year (365), and W is the mean body weight (80 kg). The annual intake per person of tomato in Egypt is 5 kg/person/year (Capmas, 2016).

In this interim, the health risk index (HRI) is considered the proportion of the estimated daily intake (EDI) to the accepted daily intake (ADI). ADI values were procured from the European Union Pesticides Database (2009). HDI illustrates if the calculated amount of pesticide residues surpasses the amount of the pesticide that can be consumed every day for the lifetime. However, HRI value that counted greater than one is considered to be critical for human health (Wang *et al*., 2005; Darko and Akoto, 2008; Ahmed *et al*., 2016).

Table 7. Level of pesticide residues in cucumber samples from different markets in Egypt

City	Market	Pesticide	A.I. detected (mg/kg)	EU MRL (mg/kg)
Ciza	Dokki	Carbendazim	0.02	0.1
Assiut	El-Zahraa	Carbendazim	0.02	0.1
		Thiophanate-methyl	0.04	0.1
		Iprodione	0.21	4
		Metalaxyl	0.02	0.5
		Abamectin	0.03	0.04
		Propamocarb	0.51	5
	El-Gomhoria	Carbendazim	0.08	0.1
		Thiophanate-methyl	0.12	0.1
		Buprofezin	0.3	0.3
		Azoxystrobin	0.1	1
		Propamocarb	1.38	5
	El-Welidia	Iprodione	0.25	4
		Metalaxyl	0.05	0.5
		Azoxystrobin	0.03	1
		Propamocarb	0.18	5
		Fenpyroximate	0.01	0.08
	El-Fath	Iprodione	0.15	4
		Metalaxyl	0.04	0.05
		Imidacloprid	0.01	1
		Azoxystrobin	0.02	1
		Chlorpyrifos	0.01	0.05
	Abnoub	Iprodione	0.02	4
		Propamocarb	0.38	5
		Emamectin	0.01	0.01
		Fenpyroximate	0.02	0.08

Data in table 8 revealed the estimated daily intake values of the pesticide residues and their corresponding health risk index in the cucumber samples. HRI value, was greater than one, indicates a potential risk to human health. Interestingly, none of HRI values were found to be greater than one. Thus none of the pesticide residues that found in selected markets considered health risk issue.

Table 8. Acceptable daily intake (ADI), estimated daily intake (EDI), and Health risk index(HRI) for pesticide residues found in the cucumber.

Pesticide	ADI (µg/kg, b.wt/day)	EDI (µg/kg, b.wt/day)	HRI (EDI/ADI)	Health risk
Carbendazim	20	0.02	0.001	No
Dithiocarbamates	7	0.008	0.001	No
Thiophanate-methyl	80	0.03	0.0004	No
Iprodione	20	0.11	0.005	No
MetaIaxyl	80	0.11	0.001	No
Abamectin	2.5	0.005	0.002	No
Propamocarb	300	0.9	0.003	No
Buprofezin	10	0.1	0.01	No
Azoxystrobin	200	0.02	0.0001	No
Fenpyroximate	10	0.005	0.0005	No
Imidacloprid	60	0.002	0.003	No
Chlorpyifos	1	0.002	0.002	No
Emamectin	0.5	0.002	0.004	No

In general, other numerous studies were carried out to investigate the pesticides residues in cucumber samples. Ibrahim *et al*., 2018 found that 17 cucumber samples exceeded the MRLs levels established by the Codex Alimentarius Commission. Further, the hazard index (HI %), that represented the long-term risk assessment was in the range of 0.014%-4.19% in cucumber samples of the ADI's. Furthermore, they stated that the highest exposure was observed for methomyl, followed by famoxadone, at 4.1869% and 0.6909% in cucumber samples of ADI, respectively. Lozowicka *et al*., 2015 demonstrated that pesticide residues found in cucumber samples (11.29% below MRL and 8.21% above MRL from the total samples). Golge *et al*., 2018 revealed that pesticide residues were detected in 13.5% of cucumber samples, however, the level were below EU MRLs. Chlorpyrifos and acetamiprid were the major contributors to hazard index. Leili *et al*., 2016 determined the residual concentrations of ethion and imidacloprid in cucumbers grown in greenhouse. They found that MRLs of ethion and imidacloprid were higher than that of Codex standard level after one hour of pesticide application while the levels of pesticides were decreased about 35 and 31% for ethion and imidacloprid, respectively one day after pesticide application which still higher than the MRL. They also confirmed that washing procedure led to about 51 and 42.5% loss in ethion and imidacloprid residues, respectively whereas peeling procedure led to highest loss of 93.4 and 63.7% in ethion and imidacloprid residues, respectively. In the same trend, Amrollahi *et al*., 2018 concluded that the lowest level of pesticide residues was carbaryl, fenpropat, and endosulfan in cucumber samples selected from greenhouses and fields, while the highest level was concerning diazinon in cucumber samples of the fields with 55.5%. Carbaryl and permethrin showed the high residue means of 0.37 and 0.72 µg/g in tomato and cucumber samples, respectively.

In conclusion, pesticide residues on cucumber were found. These pesticides are expectedly to be influenced by different factors: evaporation of the surface residue which is dependent on temperature condition, chemical or biochemical decomposition, metabolism and photolysis. The pesticide residue problem occurred probably due to misuse and/or overuse of pesticides in the environment. These contaminants which classified as moderately to

highly toxic compounds, may have adverse health effects. The suggested satisfactory solution to these problems is that countries on concerns may implement pest management measures to reduce pesticide use and adopt awareness programs to enhance food safety. In addition, the rapid dissipation of originally applied pesticide depend upon on a variety of environmental factors such as sun light, temperature which play an important role in the behavior of pesticide in the environment, as residues may not exceed the maximum levels (MRLs). The result in this study, provided important information about the current pesticide contamination status of cucumber in the studied areas and pointed an urgent need to follow up and control the use of pesticides. As the results show that pesticides may represent a public health problem.

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رصد متبقيات المبيدات فى عينات الخيار فى بعض الأسواق المصرية

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تعتبر متبقيات مبيدات الآفات في الخضروات من الأخطار المحتملة على المستهلكين ومصدر قلق على صحة الإنسان. فى هذه الدراسة, تم تحديد وتقدير متبقيات والأيض الثانوى والأيزومرات للمبيدات باستخدام جهازى LC-MS/MS و GC-MS فى عينات الخيار التى تم جمعها من 6 أسواق فى محافظتين فى مصر, هما محافظة الجيزة (الدقى) ومحافظة أسيوط (مناطق الزهراء والجمهورية والوليدية والفتح وأبنوب). أظهرت النتائج وجود 12 من متبقيات المبيدات، وقد تجاوز أحدهما قليلاً الحد الأقصى المسموح به (ثيوفينات ميثيل = 0.12 مللجم / كجم). وجد أيضا أن مبيدى إبرودايون وبروباموكارب هما الأكثر شيوعاً لمتبقيات المبيدات المرصودة. وأوضحت النتائج أيضاً عدم وجود معامل للخطر الصحى لمتبقيات المبيدات المرصودة. وبصفة عامة, يتبين من البحث ضرورة تطبيق برنامج منتظم لتحليل متبقيات المبيدات لرصد وتحديد هذه المتبقيات فى الخضروات بصورة دورية للحفاظ على سلامة الأغذية فى مصر.