Thabet et al., *Cogent Food & Agriculture* (2016), 2: 1193926
http://dx.doi.org/10.1080/23311932.2016.1193926

cogent food & agriculture

FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Chronic exposure of insecticide and fungicide as indicator of health impact in some commonly consumed leafy vegetables: Case study

Wasfi M. Thabet[1], Amr H. Shendy[1] and Sohair A. Gadalla[1*]

Received: 30 March 2016
Accepted: 20 May 2016
First Published: 26 May 2016

*Corresponding author: Sohair A. Gadalla, Central Laboratory of Residue analysis of Pesticides and Heavy Metals in Food, Ministry of Agriculture and Land Reclamation, Agricultural Research Center, Dokki, Giza, Egypt
E-mail: sohairgadalla@gmail.com

Reviewing editor:
Fatih Yildiz, Middle East Technical University, Turkey

Additional information is available at the end of the article

Abstract: Pesticide residues were monitored in selected leafy vegetables, namely (cabbage (*capitata var. alba*), lettuce (*Lactuca sativa*), molokia/Jews mallow (*Corchorus olitorius*), spinach (*Spinacia oleracea*) in the Egyptian market during 2012. A multi-residue analysis method for 103 pesticide residue monitoring (insecticides and fungicides) was employed. Among analyzed samples, 15 different pesticides were detected in 83 samples with a percentage of 54.2 of tested samples. Meanwhile, the MRLs have been exceeded in 14.5% of totally analyzed samples. The contamination percentages were observed in descending order in cabbage, lettuce, Molokai and spinach with percentages of 71.4, 44.4, and 57 and 47.8 respectively. The obtained results revealed that chlorpyrifos and profenofos were the most frequently detected pesticides. The calculated ratio of EDI to ADI was 0.004–1.5120%, indicating no risk by pointing positive detections of pesticide residues.

Subjects: Environment & Agriculture; Environmental Studies & Management; Food Science & Technology

Keywords: pesticides residues; leafy vegetables; insecticide; fungicide; ADI (acceptable daily intake); EDI (estimated daily intake); MRLs (maximum residue limits)

ABOUT THE AUTHORS

Our research group is concerned with research topics that would help estimate the common risks and hazardous to the local consumers in Egypt. Deterministic and probabilistic approaches of risk assessment studies will be applied to monitor safety of locally consumed foods that might be contaminated by various residues (pesticide, veterinary drugs, mycotoxins and trace elements). Furthermore, risk studies will be carried out to estimate baby's food safety. On the other hand, developing and establishing miniaturized analytical techniques to monitor broad scope of compounds is also a common interest to emphasize the role of regulatory authorities. In fact, the residues obtained results were part of annual regular program of local market inspection that has been carried out in our ISO-17025 Accredited laboratory using QuEChERS coupled to LC-MS/MS and GC-MS/MS. This study will be a core for further efficient evaluation to potential hazardous effects of residues and contaminants present in Egyptian foods.

PUBLIC INTEREST STATEMENT

Pesticides (insecticides, fungicides, and herbicides) are used during production and post-harvest treatment to control insects and diseases of agriculture produce. Pesticide contamination of foods can negatively affect human health. However, among different pesticides, Organophosphorous and Pyrethroid are the most predominates group used in studied plant crops (Cabbage, Spinach, Molokia and lettuce) in Egyptian markets. The detection rate in leafy vegetable samples was somewhat higher than stalk and stem vegetable samples due to the large surface area. Therefore, assessing the risk of pesticide residues in food commodities intended for human consumption is necessary. Results obtained indicated that, even with exceedance of permissible residues limits, the estimated risk exposure indicated no health hazard associated with consumption of such plants.

1. Introduction

Pesticides are used during production and post-harvest treatment of agricultural commodities (FAO/WHO, 2013). However, increased use of chemical pesticides has resulted in contamination of the environment and also caused many associated long-term effects on human health (Bhanti & Taneja, 2007). Pesticide contamination of foods can negatively affect human health since many pesticides used in agriculture have toxic effects on living organisms. Also pesticides have been associated with a wide spectrum of human health hazards such as headaches and nausea to chronic impacts like cancer, reproductive harm and endocrine disruption (Berrada et al., 2010)

A wide range of pesticides are globally used for crops protection during the cultivation of vegetables due to heavy pest infestation throughout the season crop and food (Kalara, 2003). Literature reveals that vegetables contain the residues of pesticides above their respective maximum residue limit (MRL) may pose health hazards to consumers (Bhanti and Taneja (2005).

Different pesticides (insecticides, fungicides, and herbicides) are applied to control insects and diseases of agriculture produce (Brassica). However, the predominant use of pesticides in leafy vegetables is mainly to control a wide range of lepidopteran larvae. Moreover, fungicides and insecticides are the most widely used among the different classes of pesticides, and their levels were monitored in some leafy vegetables as they reach the consumers, raw or proceed products. Organophosphorous and pyrethroid pesticides are the most frequently detected in the Egyptian market (Sohair, Almaz, Thabet, & Nabil, 2015).

Many Egyptians consume stalk, stem and leafy vegetables, such as (cabbage, lettuce, spinach, etc.). However, exceeding the MRLs were mostly found in leafy vegetables (Duck et al., 2015). The detection rate in leafy vegetable samples was somewhat higher than stalk and stem vegetable samples. In general, vegetables that have the characteristics of a large surface area compared with weight had a high rate of detection of pesticides (Hong et al., 2003). These vegetables are mainly used in the raw or cooked state. When people consume these vegetables contaminated severely by pesticides, these chemicals can cause serious health problems. Therefore, assessing the risk of pesticide residues in food commodities intended for human consumption is necessary.

Monitoring of pesticide residues in vegetables help to assess the potential risk of these products to consumers' health and provide information on the pesticides that have been used on the field crops. In recent years, there has been an increase in violation rates of the MRLs and incidents of misuse of pesticides (Payá et al., 2007). Thus, Leafy vegetables have been given a lot of attention in monitoring programs since most of them highly consumed and are expected to contain higher pesticide residue levels than other food groups of plant origin.

MRLs have been established for agricultural products in many countries to avoid the health hazard caused by pesticide residues. Codex Alimentarious limits are the standards used in Egypt in addition of others such as European standards. Health safety limits for human health are typically expressed as acceptable daily intake (ADI). The standard method to evaluate human exposure is based on the average consumption per person per day (WHO, 1997).

Many literatures have analyzed pesticide residues in various foods, with different analytical methods. However, the "Quick, Easy, Cheap, Effective, Rugged and Safe" (QuEChERS) method, is a simple sample preparation methodology for pesticide multi-residue analysis that was first reported in 2003 (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003) and is considered the most recently applied method that has been employed in the current study. However, leafy vegetables contain interfering matrix such chlorophyll, pigments that may need more extensive validation studies.

The aim of the present study is to analyze the presence of 103 pesticides, registered by local authorities and commonly used on four leafy vegetables (cabbage, lettuce, molokia and spinach) to check their compliance with existing regulations (Codex Alementarious and EU Standards). The results of the monitoring program in combination with food consumption data were taken into consideration to evaluate whether the estimated daily intake (EDI) of pesticides through the studied leafy vegetables consumed by the local inhabitants is a cause of toxicological concern according to the recommended dose by the Food and Agriculture Organization and World Health Organization (WHO). The results can be used when designing future control programs for this region and taking preventive actions to minimize human health risks

2. Materials and methods

2.1. Sampling

A total of 83 samples of four commonly leafy vegetables of cabbage (*capitata var. alba*), lettuce (*Lactuca sativa*), molokia/Jews mallow (*Corchorus olitorius*), spinach (*Spinacia oleracea)* were collected during 2012 from 16 markets located in five region including Greater Cairo, Delta region, northern upper Egypt, Alexandria and Ismailia as illustrate in Table 1. One unit of cabbage, and 1–2 kg of each of spinach, lettuce, molokia samples were subjected for analysis according to CommissionCodex Alimentariuos, CAC (1993). Sample preparation was performed according to the generally recommended method of sampling to achieve a representative part of the material to be analyzed. Analysis of samples were carried out either immediately upon their arrival to the laboratory or stored at 0–5°C for no longer than 2 days before analysis.

2.2. Selection of pesticides and analytical method

A survey of 103 pesticides commonly used in Egypt; comprising Acaricide (2), fungicide (31), herbicide (8) and insecticide (62) in 83 leafy vegetables was carried out as will described below.

Acaricides involve bromopropylate and hexythiazox. Fungicides include benalaxyl, bupirimate, carbendazim, chlorothalonil, cymoxanil, cyprodinil, diniconazole, famoxadone, fenarimol, fenhexamid, fludioxonil, flusilazole, flutolanil, hexaconazole, iprodione, metalaxyl, ortho-phenylphenol, penconazole, procymidone, propamocarb, propiconazole, pyrazophos, tebuconazole, tetraconazole, thiabendazole, thiophanate-methyl, tolclophos-methyl, Tolylfluanid, triadimefon, triadminol, vinclozolin. Herbicide, (8), Atraton, Atrazine, Bentazone, Diflufenican, Metribuzin, Pendimethalin, Thiobencarb, Trifluralin Insecticide,(62) Abamectin, Acephate, Acetamiprid, Aldicarb, (metabolites-Aldicarb Sulphone, Aldicarb Sulphoxide), Amitraz, Azinophos-ethyl, Azinphos-methyl, Bendiocarb, Bifenthrin, Buprofezine, Cadusafos, Carbaryl, Carbofuran, Carbosulfan, Chlorfenapyr, Chlorpyrifos, Chlorpyrifos-methyl, Cyanophos, Cyfluthrin, Cyhalothrin-L, Cypermethrin, Deltamethrin, Diazinon, Dimethoate, Esfenvalerate, Ethion, Ethoprophos, Fenitrothion, Fenpropathrin, Fenpyroximate, Fenthion, Fenvalerate, Indoxacarb, Lambda-cyhalothrin, Malaoxon, Malathion, Methamidophos, methidathion, Methiocarb, Methomyl, Monocrotophos, Omethoate, Oxamyl, Permethrin, Phenthoate, phosphamidon, Pirimicarb, Pirimiphos-ethyl, Profenofos, Prothiofos, Pymetrozine, Spinosad-A, Spinosad-D, tau-fluvalinate, Tefluthrin, Tetradifon, Thiacloprid, Thiamethoxam, Thiodicarb, Triazophos.

A modified QuEChERS analytical method was applied (Anastassiades, Lehotay, & Stajnbaher, 2002; Aysal, Rpa, Lehotay, & Cannavan, 2007; Sohair, Wasfi, & Emil, 2013). The procedure involves an initial extraction with acetonitrile followed by an extraction/ partitioning step after the addition of a salt mixture. An aliquot of the raw extract is then cleaned up by dispersive solid-phase extraction (d-SPE). The final extract in acetonitrile is directly amenable to determinative analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS), while quantification was performed using an internal standard, which was added directly before injection in gas chromatography tandem mass spectrometer (GC-MS/MS).

Table 1. Number of collected samples of leafy vegetables from different region during 2012

Region	No of samples
Greater Cairo (Cairo, Giza and Qalyubia)	
6th Of October Market	3
Agouza Market	2
Bolak Market	1
Giza Market	5
Helwan Market	3
Qalyobiya Market	13
Shubra Market	3
	30
Delta Region (Sharqia, Monufia and Gharbia)	
Monufia Market	6
Sharqia Market	10
Tanta Market	3
	19
Northern Upper Egypt (Minya, Beni Suef and Faiyum)	
Minya Market	1
Beni Suef Market	10
Fayoum Market	12
	23
Alexandria	
Alexandria Market	2
Nobaria Market	4
	6
Ismailia	
Ismailia Market	5
Total	83

3. Instrumentations and conditions

3.1. LC-MS/MS analysis

Agilent 1200 series liquid chromatography system equipped with Applied Biosystems (API 4000 Qtrape) tandem mass spectrometer with electrospray ionization (ESI) interface. Separations were achieved using a C18 column ZORBAX Eclipse XDB C18 (4.6 mm × 150 mm, 5 μm). The injection volume was 25 μL. A gradient elution program was set at flow rate of 0.3 ml/min, in which one reservoir contained 10 mM ammonium formate solution in methanol/water (1:9, v/v) and the other one contained methanol. The ESI source was used in the positive ion mode, and nitrogen was used as nebulizer gas, curtain gas, heater gas and collision gas according to manufacturer's recommendations. The source temperature was 300°C and an ion spray potential of 5500 V. Declustering potential and collision energy were optimized using a Harvard apparatus syringe pump. The Multiple Reaction Monitoring Mode (MRM) was used in which one MRM was used for quantification (quantifier peak) and others were used for confirmation (qualifier peaks).

3.2. GC-MS/MS analysis

Agilent Gas Chromatograph 7890A equipped with tandem mass spectrometer 7000B Quadrupole, EI source was used to perform analysis by using HP-5MS 5% phenyl methyl siloxane capillary column (30 m × 0.25 mm × 0. 25 μm). Samples were injected in a splitless mode and helium was used as carrier gas (1.8 ml/min). Injector temperature was 250°C, transfer line temperature was 285°C, ion source temperature was 300°C and quadrupole temperature was 180°C. The GC oven temperature was programmed to initially held at 70°C for 2 min then increased to 150°C at 25°C/min (held for 0 min), and raised to 200°C at the rate of 3°C/min (held for 0 min), then went up from 200 to 280°C at 8°C/min (held for 10 min). This resulted in a total run time of 42 min and complete separation of all the analytes.

3.3. Reagents and reference standards
All pesticide reference materials were obtained from Dr Ehrenstorfer Gmbh (Germany) with purity of at least 98%. All other chemicals and reagents were of HPLC grade and were obtained from Sigma–Aldrich (USA). Ultra-pure water was obtained using a Milli-Q UF-Plus system Millipore (Germany) with a resistivity of at least 18.2 MΩ cm at 25°C and TOC below 5 ppb.

3.4. Method performance
Sample preparation and analytical methods were validated in terms of of linearity, repeatability, limits of detection (LOD), limits of quantification (LOQ) and percentage recovery. The linearity of the standard curves was injected at 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 μg/L concentrations. All standards were injected three times ($n = 3$). The LODs were measured as the analyzed concentration based on a signal-to-noise ratio of 3 and the LOQ was defined as 3.3 × LOD. The percentage recovery was determined by spiking blank samples with appropriate aliquot of the standard pesticides. The average recoveries of tested pesticides at different concentration levels varied between 70.0 and 120.0%. The reproducibility expressed as relative standard deviation (RSD) was less than 25.0%. The limit of quantitation at 0.01 mg/kg was identified. The measurement uncertainty expressed as expanded uncertainty and in terms of RSD (at 95% confidence level) is lower than the default value set by the EU (±50%) SANCO/12571/2013 (XXXX).

4. Estimated daily intake calculation
Assessment of consumer's exposure to pesticide residues in leafy samples was via EDI calculation. The EDIs were calculated according to the equation presented below and expressed as percentages of the ADI and the body weight figure is 60 kg (WHO, 1997). The exposure to pesticide residues was calculated on a total of detected residues using the data obtained from monitoring and food consumption assumptions set by (WHO/GEMS/Food Cluster diets, 2012) quotes the per capita food consumption rate in Egypt as G06.

In the case of non-availability of consumption rate of commodity such molokia the consumption level for a similar food (spinach) is used (WHO, 1997). The selected consumption rate of cabbage is 20.6 g/day and 9.4 g/day for each of lettuce, molokai and spinach. The EDI was compared to corresponding ADI values of WHO/FAO database (FAO/WHO, XXXX).

The study included 103 compounds in 83 samples of leafy vegetables collected from different governorates. Results below the LODs of analytical methods used for intake calculations were taken as LOD values. The total exposure to a given pesticide residue, was obtained by summing exposures from all residue pesticide/food combinations. Values of ADI are elaborated by Joint FAO/WHO Meeting on Pesticide Residues and the European Food Safety Authority (EFSA) of the European Union (EU) (EFSA, 2008).

$$\text{EDI } \mu\text{g/kg body weight(b.w.)/day} = \text{leafy vegetables consumption (g/kg b.w./day)} \times \text{residue (mg/kg)}$$

5. Results and discussion
The developed multi-residue method (QuEChERS) was employed for anlaysis of pesticide residues in vegetables and other food commodities (Anastassiades et al., 2003; Qozowicka, Jankowska, & Kaczy, 2012). The selected modified method was applied to monitor 103 of registered pesticides including, 2 acaricide, 31 fungicide, 8 herbicide, 62 insecticide. Great concern was paid to fungicides and insecticides because of their common and extensive use in Egypt for pest control. A total of 83 samples

from different four types of the most commonly consumed leafy vegetables; cabbage, lettuce, molokia and spinach were collected from different governorates around Egypt during 2012.

5.1. Monitoring results

A total of (15) pesticide residues were detected and quantified in the analyzed samples. Data in Table 2 shows the ranges of the detected pesticide residues, their MRLs as well as the LOQ's and the instrument used in analysis. Both techniques of LC-MS/MS and GC-MSD were employed for quantification with LOQs of 0.01 mg/kg. The obtained results compared with the MRLs of codex as it set, in case of lack of codex MRLs of the European commission figures could be used. Table 3 summarizes the contamination and violation rates for each plant crop. The results represented total contamination and violation % of 54.2 and 14.5, respectively. Whereas, the most contaminated crops in descending order were, cabbage, molokia, spinach and lettuce with a percentages of 71.4, 57.1, 47.8, and 44.4, respectively. No violation was observed in cabbage while, 21% of violations were detected in molokia and spinach and 5.6% in lettuce. It deserves to mention that no correlation or trend be observed between the pesticide residues content and the season of lettuce crop.

Similar contamination % was noticed upon comparing the results of monitoring for the year of 2011 and 2012. The results revealed that the studied crops (lettuce, molokai, spinach) were contaminated by 50, 55.6 and 55.6% respectively at 2011 and 44.4, 57.1 and 47.8% at 2012. No violation was observed in lettuce and spinach, while molokia exhibited 5.5% violation in 2011 (Sohair et al., 2013). The violation rates indicated misuse and intensive pesticides spray.

Based on the sits of sampling, it was found that the percentages of contaminations ranged between 40% (Ismailia) followed by Great Cairo governorates (37%), Alexandria (33%) then 21.7% and 15% for northern upper and Delta regions respectively. Highest violation has been observed in delta region including, Sharqia, Monufia and Gharbia which are rural governorates.

5.2. Results based on detected pesticides

The frequency percentages of the fifteen detected pesticide residues in descending order were, (Table 4)chlorpyrifos, profenofos (35, 15%, respectively), carbendazim and cypermethrin (10% for each), atrazine and lambda-cyhalothrin (6.7% for each), phenthoate (3.3%), acetamiprid, diazinon, diphenylamine, malathion, metalaxyl, methomyl, omethoate, propamocarb (1.7% for each), Figure 1(a) and (b). Organophosphorus insecticides were the most detected pesticides such as chlorpyrifos which has a broad spectrum activity and authorized for application on many plant crops. Cholinesterase inhibition is the mode of action of chlorpyrifos and is the cause of potential toxicity in humans (Oliver, Bolles, & Shurdut, 2000). Chlorpyrifos was the highest detected residue in many other studies. It was the highest detected and violated pesticide in 28 vegetable samples collected from Polish farmers' fields (Qozowicka et al., 2012).

It was noted that combination of two active substances like chlorpyrifos and cypermethrin is a very often-used insecticide (Rosa, Raquel, Beatriz, & Simal, 2008), however chlorpyrifos plus cypermethrin affects both the axonic and synaptic transmission of the nerve impulses in the nervous system of insects.

In the current work, based on the detected pesticide groups, it has been observed that insecticide residues represented the majority of the detected compounds, (10 compounds) with percentage of 78.3% followed by fungicides (4 compounds) with percentage of 15%. However. The detected insecticides were of 4 chemical classes (carbamate, neonicotinoid organophosphorus and, pyrethroids) Figure 1(a). These results were somewhat in agreement to the previously published report by Selim, El-Saeid, & Al-Dossari, (2011) where the majority of detected pesticide residues in leafy vegetables collected from Riyadh, Saudi Arabia throughout 2 years (2007–2008) were insecticides including, organochlorins, organophosphorus, pyrethroids and carbamates. (Selim et al., 2011). However, it is worthy to mentioning that there is no organochlorins detected in Egyptian leafy vegetables. On the other hand, the obtained results were in contrast with Rose M. et al., who observed more positives

cogent • food & agriculture

Table 2. Pesticide residues detected in leafy vegetables samples collected from local markets during 2012

Compound	Samples	Freq*	Freq**	Min	Max	Mean	MRL mg/kg		Violated comp	LOQ	instrument
				mg/kg	mg/kg	mg/kg	Codex	EU		mg/kg	
Acetamiprid	Spinach	1	0	0.14	0.14	0.14	–	5.00	–	0.01	GC–MS MS
Atrazine	Lettuce	1	1	<LOQ	<LOQ	<LOQ				0.02	LC–MS MS
	Molokia	3	2	0.02	0.02	0.02	–	0.05*	–		
Carbendazim	Cabbage	1	1	<LOQ	<LOQ	<LOQ				0.01	LC–MS MS
	Lettuce	3	0	0.21	4.8	1.87	5		–		
	Molokia	2	0	0.02	0.54	0.28	–	0.1*	1		
Chlorpyrifos	Cabbage	1	0	0.01	0.01	0.01	1			0.01	LC–MS MS
	Lettuce	3	2	0.02	0.02	0.02	–	0.05	–		
	Molokia	9	3	0.01	0.15	0.28	–	0.05*	2		
	Spinach	8	2	0.01	2.28	0.44	–	0.05*	3		
Cypermethrin	Lettuce	1	1	<LOQ	<LOQ	<LOQ	0.7				
	Molokia	3	2	0.06	0.06	0.06	0.7		–		
	Spinach	2	0	0.03	0.22	0.13	0.7		–		
Diazinon	Molokia	1	1	<LOQ	<LOQ	<LOQ				0.01	GC–MS MS
Diphenylamine	Lettuce	1	0	0.02	0.02	0.02	–	0.05	–	0.01	LC–MS MS
Lambda-Cyhalothrin	Molokia	2	1	0.01	0.01	0.01	–	0.5	–	0.01	LC- MS MS
	Spinach	2	0	0.22	0.25	0.24	–	0.50	–		
Malathion	Lettuce	1	0	0.02	0.02	0.02	–	0.5	–	0.01	GC–MS MS
											LC–MS MS
Metalaxyl	Lettuce	1	0	0.03	0.03	0.03	2		–	0.01	GC–MS MS
											LC–MS MS
Methomyl	Molokia	1	0	0.03	0.03	0.03	–	0.05	–	0.01	GC–MS MS
											LC–MS MS
Omethoate	Spinach	1	0	0.02	0.02	0.02	–	0.02*	–	0.01	GC–MS MS

Freq* no of detection for < LOQ and > LOQ; Freq** of samples contaminated with levels < LOQ; EU-MRLs compared in case of Codex lack; violation consider in case of MRL lack/banned in Codex and EU.

fungicides detected at larger concentrations than insecticides, especially for lettuces in Spain

Table 3. Number of analyzed samples, free, contaminated and violated samples collected from markets during 2012

Leafy Veg.	No. of samples	Free samples		Samples with LOQ levels		Contaminated samples*		Violated samples	
		No.	%	No.	%	No.	%	No.	%
Cabbage	14	4	28.6	-	-	10	71.4	-	-
Lettuce	18	10	55.6	1	5.6	8	44.4	1	5.6
Molokia	28	12	42.9	4	14.3	16	57.1	6	21.4
Spinach	23	12	52.2	2	8.7	11	47.8	5	21.7
Total	83	38	45.8	7	8.4	45	54.2	12	14.5

Contaminated samples* include samples contaminated with levels <LOQs.

Table 4. Pesticide residues detected according to the functional classification and the their frequencies percentages

Pesticide type	Compound	Substance group	No of detection	freq %
Insecticide	Acetamiprid	Neonicotinoid	1	1.7
	Chlorpyrifos	Organophosphate	21	35.0
	Cypermethrin	Pyrethroid	6	10.0
	Diazinon	Organophosphate	1	1.7
	Lambda-Cyhalothrin	Pyrethroid	4	6.7
	Malathion	Organophosphate	1	1.7
	Methomyl	Carbamate	1	1.7
	Omethoate	Organophosphate	1	1.7
	Phenthoate	Organophosphate	2	3.3
	Profenofos	Organophosphate	9	15.0
			47	78.3
Herbicide	Atrazine	Triazine	4	6.7
			4	6.7
Fungicide	Carbendazim	Benzimidazole	6	10.0
	Diphenylamine	Amine	1	1.7
	Metalaxyl	Phenylamide	1	1.7
	Propamocarb	Carbamate	1	1.7
			9	15
			60	

markets, which emphasized that use of pesticides is linked to environmental conditions (Rosa et al., 2008).

The obtained data shows that, the banned residues of phenthoate was detected twice with mean values of 0.01 and 0.04 mg/kg in molokia and spinach, respectively. However, data from previous study at 2011 showed higher detection of phenthoate with a percentage of 2.3% in vegetable samples indicating continuous phasing out of phenthoate from Egyptian markets. It should be noted that, registration for phenthoate was recently canceled out in Egypt since 2010.

The explanation for the highly detected pesticide residues in leafy vegetables might be due to intensive use of pesticide and highly deposited amounted of the applied pesticides on the broad leafs of such vegetables. Moreover, some leafy vegetables such as molokia, lettuce cultivated in Egypt beside fields of other crops so that may get contaminated with the extensive spray of pesticides. (Dogheim, El-Marsafy, Gad Alla, Khorshid, & Fahmy, 2004; Sohair et al., 2013).

Ashutosh, Purushottam, Srivastava, Lohani, & Laxman, (2011) reported the results of pesticide residue monitored in samples of vegetable collected from Lucknow City, India, using QuEChERS method. They found 23 pesticide residue in the samples with the range of 0.005–12.35 mg/kg and some of the detected pesticides were organochlorine which were worldwide banned. Also, 180 samples of 15 leafy vegetables type monitored for pesticide residue from Korean wholesale and traditional markets during 2010. Seven pesticides were detected with low contamination rate of 6.7% and exceeding rate of 0.6%, Hyum et al., (2010)

5.3. Chronic exposure assessment

Table 5 illustrated the results of estimation of chronic dietary exposure to pesticide residues from studied leafy vegetables for both individual and the common action mode groups of pesticides. Chronic exposure assessment was carried out for the general Egyptian population (60 kg b.w) using

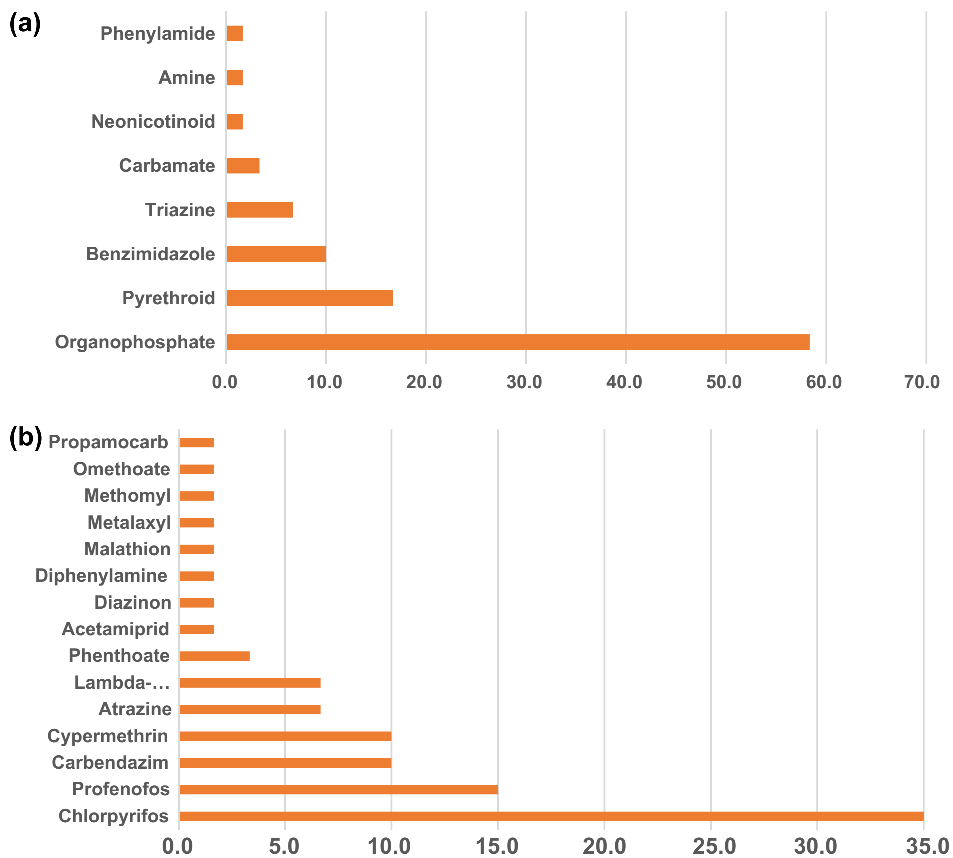

Figure 1. The percentages of detected pesticide residues: (a) based on chemical groups and (b) based on individual pesticide residues.

average detected pesticide residue levels and mean consumption and compared to the ADI according to the guideline of WHO (Guidelines WHO, 1997). As shown in Table 5 the calculated EDI did not exceed the ADI certainly. Overall results obtained from pesticide residue assessment performed in leafy vegetables samples indicate no risk by pointing positive detections of pesticide residues.

However, Godfred & Osei, (2008) reported a health risks e associated with OP's such as methyl-chlorpyrifos, ethyl-chlorpyrifos, and omethoate, dichlorvos and monocrotophos in some vegetables samples collected from Ghanaian market.

Cumulative chronic dietary exposure to acetylcholinesterase inhibitors like pesticides of organophosphorus (OP's) group (EPA, 2006) and sodium channel modulators pesticide group (pyrethroid) (EPA, 2011) was evaluated. The % ADI of (OP's) estimated for the adults were below 2% ranging from 0.027 to 1.078% ADI which unlikely constitute any risk. It has been found that the calculated cumulative risk for sodium channel modulator compounds (pyrethroid) was ranging from 0.003 to 0.776% ADI.

The cumulative assessment of organophosphorous insecticides in leafy vegetable samples was higher than that of pyrethroid compounds, but in all cases was below 4% ADI. The potential chronic dietary exposure for adults' general population at mean consumption level was 3.785 and 0.873% ADI for organophosphorous and pyrethroid, respectively. The long-term consumer exposure to individual pesticide residues (e.g. diazinon) does not exceed the % of the ADI as shown in Table 5. The total risk of chlorpyrifos in all analyzed commodities yielded, 0.123 μg/kg b.w./day (1.23% ADI) for adults with high level of 0.711% ADI in spinach.

Table 5. Estimation of chronic dietary exposure to pesticide residues for Leafy vegetables in 2012

Pesticide group	Active substance	Samples	Average residues	Acceptable daily intake (ADI)	General population (60 kg) (mean)	
			mg/kg	μg/kg b.w./day	μg/kg b.w. /day	ADI %
Amine	Diphenylamine	Lettuce	0.02	75	0.0032	0.004
Benzimidazole	Carbendazim	Cabbage	0.01	20	0.0034	0.017
		Lettuce	1.87	20	0.3023	1.512
		Molokia	0.28	20	0.0453	0.226
Carbamate	Methomyl	Molokia	0.03	2.5	0.0049	0.194
	Propamocarb	Lettuce	0.92	290	0.1487	0.051
Neonicotinoid	Acetamiprid	Spinach	0.14	70	0.0226	0.032
Organophosphate	Chlorpyrifos	Cabbage	0.01	10	0.0034	0.034
		Lettuce	0.02	10	0.0032	0.032
		Molokia	0.28	10	0.0453	0.453
		Spinach	0.44	10	0.0711	0.711
	Diazinon	Molokia	0.01	0.2	0.0016	0.808
	Malathion	Lettuce	0.02	30	0.0032	0.011
	Omethoate	Spinach	0.02	0.3	0.0032	1.078
	Phenthoate	Molokia	0.01	3	0.0016	0.054
		Spinach	0.04	3	0.0065	0.216
	Profenofos	Lettuce	0.05	30	0.0081	0.027
		Molokia	0.53	30	0.0857	0.286
		Spinach	0.14	30	0.0226	0.075
						3.785
Phenylamide	Metalaxyl	Lettuce	0.03	0.08	0.0049	0.006
Pyrethroid	Cypermethrin	Lettuce	0.01	0.05	0.0016	0.003
		Molokia	0.06	0.05	0.0097	0.019
		Spinach	0.13	0.05	0.0210	0.042
	L-Cyhalothrin	Molokia	0.01	0.005	0.0016	0.032
		Spinach	0.24	0.005	0.0388	0.776
						0.873
Triazine	Atrazine	Lettuce	0.01	0.02	0.0016	0.008
		Molokia	0.02	0.02	0.0032	0.016

Although these results pointed a negligible risk associated with the exposure, a special precaution should be taken with the possible aggregate exposure to these chemicals from multiple sources of nutrition and domestic use of pesticides as previously reported (Qozowicka et al., 2012).

6. Conclusion

It was also concluded that insecticides including organophosphorus and pyrethroids still the major residues detected with higher percentages of contamination and violation in Egypt. Thus, regular monitoring of pesticide residues in agricultural crops, both during cultivation and in markets is required to prevent, control and minimize health risks. In addition the obtained results clearly indicate the actual situation of the misuse of insecticide which may affect in turn the consumer's health at long period of exposure.

Funding
The authors received no direct funding for this research.

Competing Interests
The authors declare no competing interests.

Author details
Wasfi M. Thabet[1]
E-mail: wasfqcap@hotmail.com
Amr H. Shendy[1]
E-mail: amr_shendy@yahoo.com
Sohair A. Gadalla[1]
E-mail: sohairgadalla@gmail.com
[1] Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food, Ministry of Agriculture and Land Reclamation, Agricultural Research Center, Dokki, Giza, Egypt.

Citation information
Cite this article as: Chronic exposure of insecticide and fungicide as indicator of health impact in some commonly consumed leafy vegetables: Case study, Wasfi M. Thabet, Amr H. Shendy & Sohair A. Gadalla, *Cogent Food & Agriculture* (2016), 2: 1193926.

References

Anastassiades, M., Lehotay, S. J., & Stajnbaher, D. (2002). *Quick, easy, cheap, effective, rugged, and safe (QuEChERS) approach for the determination of pesticide residues* (pp. 231–241). In 18thAnnual waste testing and quality symposium proceeding, Arlington, VA.

Anastassiades, M., Lehotay, S. J., Stajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solidphase extraction for the determination of pesticide residues in produce. *Journal of AOAC International, 86*, 412–431.

Ashutosh, K. S., Purushottam, T., Srivastava, M. K., Lohani, M., & Laxman, P. S. (2011). Monitoring of pesticide residues in market basket samples of vegetable from Lucknow City, India: QuEChERS method. *Environmental Monitoring and Assessment, 176*, 465–472.

Aysal, P., Rpa, A. A., Lehotay, S. J., & Cannavan, A. (2007). Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. *Journal of Environmental Science and Health Part B, 42*, 481–490. http://dx.doi.org/10.1080/19312450701392490

Berrada, H., Fernandez, M., Ruiz, M. J., Molto, J. C., Manes, J., & Font, G. (2010). Surveillance of pesticide residues in fruits from Valencia during twenty months (2004/2005). *Food Control, 21*, 36–44. http://dx.doi.org/10.1016/j.foodcont.2009.03.011

Bhanti, M., & Taneja, A. (2005). Monitoring of organochlorine pesticide residues in summer and winter vegetables from Agra, India––A case study. *Environmental Monitoring and Assessment, 110*, 341–346. http://dx.doi.org/10.1007/s10661-005-8043-6

Bhanti, M., & Taneja, A. (2007). Contamination of vegetables of different seasons with organophosphorous pesticides and related health risk assessment in northern India. *Chemosphere, 69*, 63–68. http://dx.doi.org/10.1016/j.chemosphere.2007.04.071

CommissionCodex Alimentariuos. (1993). *Portion of commodities to which maximum residue limits apply and which is analyzed CAC/GL 41-1993* (Joint FAO/WHO food standards program, Vol. 2, p. 391).

Dogheim, S. M., El-Marsafy, A. M., Gad Alla, S. A., Khorshid, M. A., & Fahmy, S. M. (2004). Pesticides and heavy metals levels in Egyptian leafy vegetables and some aromatic medicinal plants. *Food Additives and Contaminants, 21*, 323–330. http://dx.doi.org/10.1080/02652030310001656361

Duck, W. P., Kwang, G. K., Eun, A. C., Gyeong, R. K., Tae, S. K., Yong, S. Y., … Bae, S. C. (2015). Pesticide residues in leafy vegetables, stalk and stem vegetables from South Korea: A long-term study on safety and health risk assessment, *Food Additives and Contaminants Part A, 33* 1–14.

EFSA. (2008, July). *Report on the toxicological reference values for the active substances in plant protection products* (Web report No. 1). Author.

EPA. (2006, July 31). Organophosphorus cumulative risk assessment––2006 update. *U.S. Environmental Protection Agency Office of Pesticide Programs*. Retrieved March 12, 2014, from http://www.epa.gov/oppsrrd1/cumulative/2006-op/op_cra_main.pdf

EPA. (2011). Pyrethrins/pyrethroid cumulative risk assessment. *U.S. Environmental Protection Agency Office of Pesticide Programs*. Retrieved March 12, 2014, from http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2011-0746-0003

FAO/WHO. (2013). *FAO/WHO*. Retrieved January, 2013, from http://www.codexalimentarius.net

FAO/WHO database. (XXXX). Retrieved from http://www.fao.org/faowhocodexalimentarius/standards/pesticide-mrls/en/

Godfred, D., & Osei, A. (2008). Dietary intake of organophosphorus pesticide residues through vegetables from Kumasi. *Ghana, Food and Chemical Toxicology, 46*, 3703–3706.

Hong, M. K., Hwang, I. G., Choi, D. M., Lee, K. B., Oh, G. S., Suh, J. H. … Lee, J. (2003). Monitoring of pesticide residues in foods. *Annual Reports in KFDA., 7*, 104–111.

Hyum, H. N., Young, S. P., Kyung, W. K. P., Kwang, H. L., Jae, Y. L., Kyung, W. Y. … Kee, S. K. (2010). Monitoring of pesticide residues in leafy vegetables collected from wholesale and traditional markets in Cheongju. *The Korean Journal of Pesticide Science, 14*, 381–393.

Kalara, R. L. (2003). Assessment of human exposure of pesticide residues through food, and water and other sources in India (pp. E1–9). In Proceedings of symposium on risk assessment of pesticide residues in water and food (IL SI Washigton DC). ITRC Lucknow and ICMR New Delhi India on October 28–29.

Oliver, G. R., Bolles, H. G., & Shurdut, B. A. (2000). Chlorpyrifos: Probabilistic assessment of exposure and risk. *Neurotoxicology, 21*, 203–208.

Payá, P., Anastassiades, M., Mack, D., Sigalova, I., Tasdelen, B., & Oliva, J. (2007). Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. *Analytical and Bioanalytical Chemistry, 389*, 1697–1714. http://dx.doi.org/10.1007/s00216-007-1610-7

Qozowicka, B., Jankowska, M., & Kaczy, N. P. (2012). Pesticide residues in Brassica vegetables and exposure assessment of consumers. *Food Control, 25*, 561–575. http://dx.doi.org/10.1016/j.foodcont.2011.11.017

Rosa, M. G., Raquel, R. O., Beatriz, C. G. J., & Simal, G. N. (2008). Occurrence of fungicide and insecticide residues in trade samples of leafy vegetables. *Food Chemistry, 107*, 1342–1347.

SANCO/12571/2013. (XXXX). Guidance document on analytical quality control and validation 385 procedures for pesticide residues analysis in food and feed. Retrieved from http://ec.europa.eu/food/plant/pesticides/guidancedocuments/docs/qualcontrolen.pdf

Selim, M. T., El-Saeid, M. H., & Al-Dossari, L. M. (2011). Multi-residues analysis of pesticides using gas chromatography mass spectrometry: I-leafy vegetables. *Research Journal of Environmental Sciences, 5*, 248–258.

Sohair, A. G. A., Wasfi, M. T., & Emil, Y. S. (2013). Monitoring and risk assessment of pesticide residues in some Egyptian vegetables. *Middle East Journal of Applied Sciences, 3*, 216–230. ISSN: 2077-4613.

Sohair, A. G. A., Almaz, M. M., Thabet, W. M., & Nabil, M. M. (2015). Evaluation of pesticide residues in some Egyptian fruits. *International Journal of Environment*, 4, 87–97.

Sukesh, N. S., Vasudev, K., & Vishnu, V. R. M. (2012). Quantification of organophosphate insecticides and herbicides in vegetable samples using the "Quick Easy Cheap Effective Rugged and Safe" (QuEChERS) method and a high-performance liquid chromatography–electrospray ionization mass spectrometry (LC–MS/MS) technique. *Food Chemistry, 132*, 1574–1584.

WHO. (1997). Guidelines for predicting dietary intake of pesticide residues (revised) global environment monitoring system–food contamination monitoring and assessment programme (GEMS/Food) in collaboration with Codex Committee on pesticide residues. *Programme of Food Safety and Food Aid*, 1–44.

WHO/GEMS/Food Cluster diets. (2012). Retrieved from https://extranet.who.int/sree/Reports?op=vs&path=/WHO_HQ_Reports/G7/PROD/EXT/GEMS_cluster_diets_2012&userid=G7_ro&password=inetsoft123