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Research Article

Dietary Intake of Potential Pesticide Residues in Tomato Samples Marketed in Egypt

¹Mohamed Ahmed Ibrahim Ahmed, ²Tarek Abd Elaliem Abd El Rahman and ²Nasr Sobhy Khalid

¹Department of Plant Protection, Faculty of Agriculture, Assiut University, 71526 Assiut, Egypt

²Central Agricultural Pesticides Laboratory, Agricultural Research Center, Dokki, 12618 Giza, Egypt

Abstract

Background and Objective: Pesticide residues in vegetables and fruits are considered impact issue in Egypt. The aim of this study is to generate an essential method to evaluate the pesticide residues in tomato samples in random market in Egypt. **Methodology:** Herein, a total of 38 pesticides (12 organochlorines, 6 pyrethroids and 20 organophosphorous) in 16 different tomato samples collected from 8 local markets in 5 major cities in Egypt were monitored by gas chromatography with an electron capture detector (GC-ECD) for organochlorines and pyrethroids, with a flame photometric detector (GC-FPD) for organophosphorous pesticides. **Results:** Pesticide residues were found in 13 samples and 7 samples were higher than the Maximum Residue Levels (MRLs). However, the most frequently found pesticides were heptachlor-epoxide, P,P'-DDE and profenofos, followed by gamma-HCH and pirimiphos-methyl. Furthermore, washed with a NaCl-saturated solution was the most effective method for reducing the pesticide residues. **Conclusion:** Furthermore, the health risk index for heptachlor-epoxide was the greatest, which may be due to its physiochemical properties. A potential regular pesticide residues program in vegetables should be conducted to protect consumer health.

Key words: Pesticide residues, QuEChERS, gas chromatography, MRL, hazard risk index

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Corresponding Author: Mohamed Ahmed Ibrahim Ahmed, Department of Plant Protection, Faculty of Agriculture, Assiut University, 71526 Assiut, Egypt
Tel: +2-01113991177 Fax: +2-088-2331384

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pesticides are considered to be essential in controlling pests in horticultural crops and insect-borne diseases¹⁻⁵. However, pesticides are used to minimize crop loss before and after the harvest process⁶ and to maximize the crop yield per acre to meet the growing demand of the people⁷. Their intensive use has led to contamination of the environment and their residue may be accumulated at a level higher than is permitted in food, particularly in fruits and vegetables^{8,9}.

Unfortunately, many pesticides are toxic, contain persistent chemicals in the environmental components and are associated with a broad spectrum of human health hazards^{10,11}. In Egypt, organochlorine and organophosphorus pesticide residues have been found in potatoes and cucumber samples. This concerns many factors, for instance the illiteracy and low levels of knowledge and awareness of the hazardous effects of pesticides to human health and to surrounding environment¹²⁻¹⁴.

Thus, it is very important to formulate numerous multiresidue methods for monitoring and detecting pesticide residues in vegetables and fruit samples to avoid possible health risks. One of the most effective methods is Gas Chromatography (GC). The GC performs well for analyzing organochlorine and organophosphorus pesticide residues, allowing several analyses of volatile pesticides with high and unique speed of analysis, excellent and efficient separation and the availability of a large scope of sensitive and selective detectors^{10,13,15}.

In this interim, QuEChERS is a (quick, easy, cheap, effective, rugged and safe) method which has been mainly applied for the extraction of different classes of pesticides. The QuEChERS method is a simple, rapid and inexpensive procedure requiring little labor and few materials, space and solvents. This method has achieved the status of official method of AOAC International^{16,17}.

To date, no reliable data are available on the level of pesticide residues in tomatoes¹⁸, one of the most important vegetable crops in Egypt with an average production of 9,956.56 thousand tons and a consumption of 6,488.92 thousand tons in 2015. Therefore, the present study seeks to provide solid baseline information on the contamination levels of OC, PY and OP pesticide residues in tomato samples marketed in Egypt using a sensitive and selective multiresidue method for the quantitative monitoring of pesticide residues with a Gas Chromatography-Electron Capture Detector (GC-ECD) for OCs and PYs and a Gas Chromatography-Flame Photometric Detector (GC-FPD) for OPs. In addition, the study

Table 1: Random markets from Egypt from which the samples were obtained

Market	City
Alzahraa	Assiut
Alwelidia	Assiut
Abo-teg	Assiut
Der-mwas	Menia
Shobra-elkhema	Kalubia
Elmaadi	Cairo
Elharam	Giza
Eldokki	Giza

also determines the hazard indices of the health risk associated with that intake of the pesticide residues in tomato samples in Egypt.

MATERIALS AND METHODS

Sample collection: A total of sixteen samples ($\approx$ three kilograms each) were collected randomly from eight local markets in five cities in Egypt between the period of May-July, 2015 (Table 1). However, the samples collected were immediately wrapped and placed in an ice container at 4°C. Furthermore, the samples were labeled by the name of the market and city and then sent to the laboratory for analysis.

Samples preparation: The sample preparation and extraction were described before by Ahmed *et al.*¹³. In brief, the samples were completely homogenized and then divided into three portions of 1 kg each following the guidelines of Codex Guide vol. 2-section 4 FAO¹⁹ as follows:

One-kilogram sample of tomato was completely homogenized and three replicates of 100 g each were taken: Two for extraction and the third kept in the deep freezer at -20°C. Extraction was performed as soon as possible.

The samples were comminuted (10 g) and then placed into a 50 mL polyethylene tube. The samples were extracted and cleaned up immediately after sampling use QuEChERS methodology²⁰ according to the description by Ahmed *et al.*¹³.

Gas Chromatography (GC): The OC and PY pesticides were analyzed on a Hewlett Packard (HP) serial 6890 gas chromatograph equipped with an electron capture detector (GC-ECD). The GC analysis was conducted on an HP-5 MS capillary column of 30 m length, 0.25 mm column ID and 0.25 μ m film thickness. The oven temperature was programmed from an initial temperature of 80°C for 1 min, increased at 30°C min⁻¹ up to 160 (2 min hold), then increased to 260°C at a rate of 3°C min⁻¹ and maintained at 260°C for 12 min. The injector and detector temperature were maintained at 300 and 320°C, respectively. Nitrogen

Table 2: Group, pesticide, t_R , LOD, LOQ, r^2 , RSD% and average recovery percentage of OC and PY pesticides in tomato samples using GC-ECD

Groups	Pesticides	t_R (min)	LOD (mg kg ⁻¹)	LOQ (mg kg ⁻¹)	r^2	RSD (%)	Average recoveries (%)
OC	Alpha-HCH	10.44	0.005	0.015	0.995	12	75.72
	Beta-HCH	11.96	0.005	0.015	0.997	10	88.53
	Gamma-HCH	13.10	0.001	0.004	0.996	11	90.51
	Heptachlor	14.46	0.010	0.030	0.995	9	87.43
	Aldrin	17.07	0.010	0.030	0.997	9	87.47
	Heptachlor-Epoxide	17.31	0.001	0.003	0.996	18	82.21
	Dieldrin	17.72	0.001	0.003	0.991	13	89.95
	P,P-DDE	18.98	0.001	0.003	0.988	7	88.52
	Endrin	19.15	0.001	0.003	0.997	16	85.64
	O,P-DDT	20.07	0.002	0.006	0.992	16	91.57
	P,P-DDD	20.50	0.002	0.006	0.991	13	94.56
	P,P-DDT	21.09	0.002	0.006	0.992	15	90.86
	Fenpropathrin	21.98	0.001	0.003	0.997	12	94.82
	Lambda-Cyhalothrin	25.02	0.001	0.003	0.995	17	92.92
PY	Permethrin	26.17	0.001	0.004	0.994	15	89.72
	Cypermethrin	28.75	0.001	0.003	0.998	12	83.11
	Fenvalerate	34.58	0.005	0.016	0.992	13	99.43
	Deltamethrin	40.96	0.001	0.003	0.996	14	84.11

OC: Organochlorine pesticides, PY: Pyrethroid pesticides, t_R : Retention time, LOD: Limits of detection, LOQ: Limits of quantification, RSD (%): Relative standard deviation percent

was used as a carrier at a flow rate of 3 mL min⁻¹. With each set of samples to be analyzed, a solvent blank, a standard mixture and a procedural blank were run in sequence to check for contamination, peak identification and quantification.

For OP pesticides, the extract was concentrated and injected into a gas liquid chromatograph equipped with a flame photometric detector (GC-FPD). The GC analysis was conducted on a PAS-1701 (Agilent, Folsom, CA) fused silica capillary column of 30 m length, 0.32 mm column ID and 0.25 μ m film thickness. The oven temperature was programmed from an initial temperature of 160 (2 min hold) to 210°C at a rate of 5°C min⁻¹, maintained at 210°C for 3 min and raised to 240°C at rate of 5°C min⁻¹, maintained at 240°C for 1 min and raised to 270°C at a rate of 20°C min⁻¹, maintained at 270°C for 10 min. The injector and detector temperature were maintained at 240 and 260°C, respectively. Nitrogen was used as a carrier at a flow rate of 3 mL min⁻¹. The hydrogen and air flow rate were 75 and 100 mL min⁻¹, respectively. The peak was identified by a comparison of the sample retention times with those of the corresponding pure standard compounds.

Method validation: The method validation was evaluated according to the guidance method validation and quality control procedures for pesticide residue analysis in Food and Feed²¹ for its repeatability, linearity, recovery, limit of detection and quantification. Linearity was evaluated by the calculation of a five-point linear plot with three replicates (Table 2 and 3), based on linear regression and the squared correlation coefficient r^2 , which should be >0.9800. The average recovery and the highest RSD% were obtained in

repeatability studies from spiked samples at three different concentrations (LOQ, 2XLOQ and 5XLOQ). For the analysis of pesticides at sub (μ g L⁻¹) levels, the recovery values between of 70 and 120% were considered as acceptable. The accuracy of the presented method was in the range of 75.22-99.43%, which was acceptable for all pesticides tested, fulfilling the recommendation of SANCO guidelines²¹. The RSD% values were less than 20% for all of the concentration levels tested. The experiments showed no interference peaks from the tomato samples matrix on the elution region of the specific pesticides. All results obtained for all compounds confirm the efficacy of the present method for the determination of multi-residue pollutants in tomato samples.

RESULTS AND DISCUSSION

The recoveries for the pesticide residues ranging from 75.22% for azinphos-ethyl to 99.43% for fenvalerate in the tomato samples demonstrated that the method used is reproducible (Table 2 and 3). However, the method was validated under the optimized condition by determining LOD and LOQ.

The data in Table 4 show the level of pesticide residues in the tomato samples. Heptachlor-epoxide was detected in three different markets: Alzahraa (0.12 mg kg⁻¹), Der-Mwas (0.12 mg kg⁻¹) and Eldokki (0.13 mg kg⁻¹). Furthermore, heptachlor-epoxide occurred in 37.5% of the tomato samples. The level of heptachlor-epoxide found exceeded the MRL set by the European Union Pesticides Database²².

The P,P'-DDE occurred in 37.5% of the tomato samples in three different markets and the concentration exceeded

Table 3: Group, pesticide, t_R , LOD, LOQ, r^2 , RSD%, and average recovery percentage of OP pesticides in tomato samples using GC-FBD

Group	Pesticides	t_R (min)	LOD (mg kg ⁻¹)	LOQ (mg kg ⁻¹)	r^2	RSD (%)	Average recoveries (%)
OP	Azinphos-ethyl	18.8	0.001	0.003	0.991	18	75.22
	Chlorpyrifos	8.47	0.001	0.003	0.988	15	86.81
	Chlorpyrifos-methyl	7.39	0.001	0.003	0.997	11	96.99
	Cadusafos	4.49	0.001	0.003	0.992	12	87.42
	Diazinon	5.77	0.001	0.003	0.981	15	98.32
	Dichlorovs	1.49	0.001	0.003	0.983	17	95.97
	Disulfoton	6.0	0.005	0.014	0.991	10	96.16
	Cyanophos	7.13	0.005	0.015	0.988	14	75.72
	Ethion	13.4	0.005	0.015	0.997	12	81.53
	Ethoprophos	3.92	0.001	0.004	0.992	13	90.51
	Fenitrothion	8.6	0.010	0.030	0.991	12	87.43
	Fenamiphos	11.8	0.010	0.040	0.992	12	87.47
	Methamidophos	1.7	0.001	0.003	0.997	12	82.21
	Phorate	4.8	0.001	0.003	0.995	13	89.95
	Phenthoate	9.3	0.001	0.004	0.994	16	88.52
	Pirimiphos-ethyl	8.07	0.001	0.003	0.995	17	85.64
	Pirimiphos-methyl	8.8	0.002	0.006	0.997	12	97.57
	Profenofos	11.6	0.002	0.006	0.996	14	94.56
	Prothiofos	10.22	0.002	0.006	0.995	12	94.86
	Triazophos	14.09	0.001	0.005	0.997	12	96.92

OC: Organophosphorus pesticides, t_R : Retention time, LOD: Limits of detection, LOQ: Limits of quantification, RSD (%): Relative standard deviation percent

Table 4: Level of pesticide residues in tomato samples from different markets in Egypt

City	Market	Pesticide	MRLs (mg kg ⁻¹)	Unwashed	Washed	Washed by NaCl-saturated solution
Assiut	Alzahraa	Gamma-HCH	0.01	0.025	0.017	0.015
		Heptachlor-Epoxide	0.0	0.12	0.046	0.029
	Alwelidia	P,P'-DDD	0.05	0.08	0.061	0.031
		P,P'-DDE	0.05	0.06	0.02	0.002
	Abo-Teg	Profenofos	10	0.1	0.06	0.03
		P,P'-DDE	0.05	0.03	0.001	ND
Menia	Der-Mwas	Heptachlor-Epoxide	0.01	0.12	0.05	0.03
		Profenofos	10	0.31	0.08	0.024
Kalubia	Shobra-Elkhema	Pirimiphos-Methyl	1	0.01	0.001	ND
		Gamma-HCH	0.1	0.01	ND	ND
Cairo	Elmaadi	Profenofos	10	0.02	ND	ND
Giza	Elharam	P,P'-DDE	0.05	0.1	0.06	0.02
	Eldokki	Heptachlor-Epoxide	0.01	0.13	0.07	0.04

MRL: Maximum residue levels (according to the European Union Pesticides Database)²²

the MRL in 2 markets: Alwelidia (0.06 mg kg⁻¹) and Elharam (0.1 mg kg⁻¹). Profenofos was detected in three markets in 6 samples and all of them were below the MRL of 10 mg kg⁻¹. γ -HCH occurred in 25% of the samples. However, the concentration of detected residue in Alzahraa market was above the MRL of 0.01 mg kg⁻¹ by 2.5-fold.

The P,P'-DDD and pirimiphos-methyl were the least frequently detected residues in all tomato samples. Their occurrences were in 0.08 and 0.01 mg kg⁻¹, respectively, in 6.25% of tomato samples for each pesticide residue.

Interestingly, washed by saturated NaCl was the most effective method in all samples (Table 4 and Fig. 1). However, the reduction percentage ranged from 40-96.67% using this method. Furthermore, the pesticide residues were not detectable after the use of both washing methods in Cairo.

From a potential health perspective, it is essential to assess the Estimate Daily Intake (EDI), which is the realistic estimate of pesticide residue calculated with the perspective

of international guidelines²³ which is expressed as microgram of pesticides per kilogram body weight per day ($\mu\text{g kg}^{-1} \text{ b.wt., day}^{-1}$) and calculated from the following equation:

$$\text{EDI} = \sum \frac{C \times F}{D \times W} \quad (1)$$

where, C is the sum of the concentration of pesticide in each location ($\mu\text{g kg}^{-1}$), F is the mean annual intake of food per person, D is number of days in a year (365 days) and W is the mean body weight (80 kg). The annual intake per person of tomato in Egypt is 97 kg/person/year¹⁸.

However, the Health Risk Index (HRI) is the ratio of the Estimated Daily Intake (EDI) to the Accepted Daily Intake (ADI) obtained from the European Union²⁴. The HDI demonstrates whether the calculated amount of pesticide residues exceeds the amount of the pesticide that can be consumed

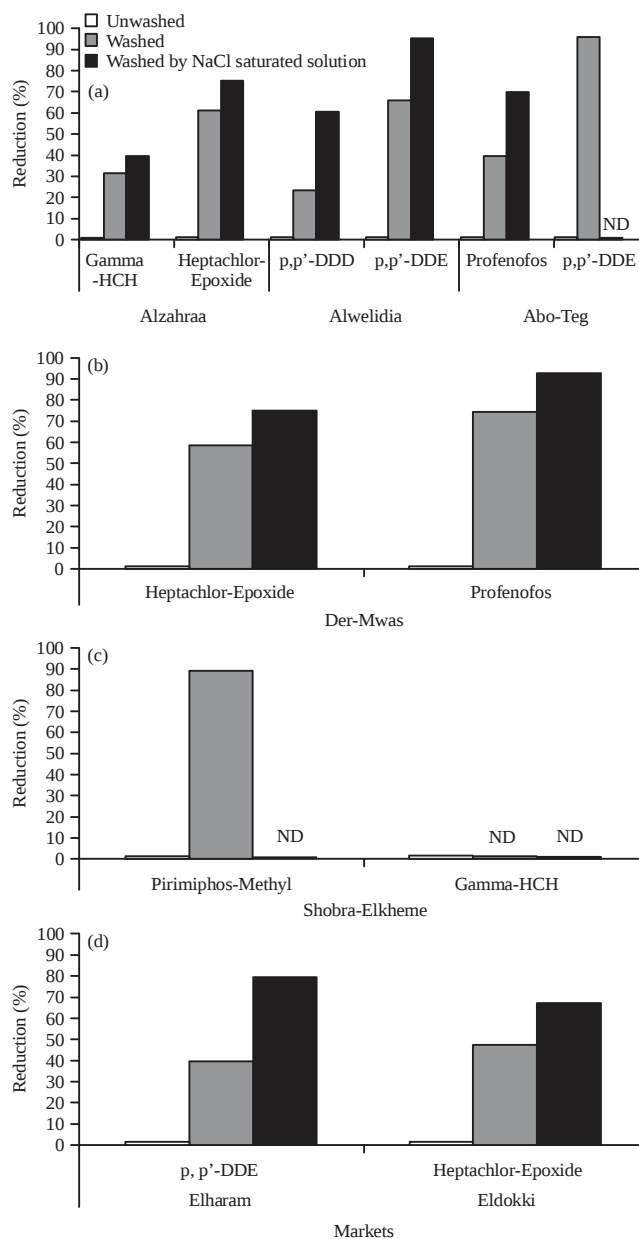


Fig. 1(a-d): Reduction percentage of pesticide residues found after using 2 methods (washed and washed by NaCl-saturated solution in four cities, (a) Assiut, (b) Menia, (c) Kalubia and (d) Giza city, Egypt

Table 5: Acceptable daily intake (ADI), estimated daily intake (EDI), and health risk index (HRI) for pesticide residues found in the tomato samples studied

Pesticide	ADI ($\mu\text{g kg}^{-1} \text{ b.wt., day}^{-1}$)	EDI ($\mu\text{g kg}^{-1} \text{ b.wt., day}^{-1}$)	HRI (EDI/ADI)	Health risk
γ -HCH	1	0.1	0.1	No
Heptachlor-Epoxide	0.1	0.5	5	Yes
P,P'-DDD	10	0.3	0.03	No
P,P'-DDE	10	0.7	0.07	No
Profenofos	30	1.0	0.03	No
Pirimiphos-methyl	4	0.03	0.008	No

every day for the life time. An HRI value greater than one is considered to be unsafe for human health^{25,26}.

Table 5 presents the estimated daily intake values of the pesticide residues and their corresponding health risk index in

the tomato samples. An HRI value greater than one indicates that the EDI exceeds the ADI values and it is considered a potential risk to human health. Alarming, the highest HRI value was found for heptachlor-epoxide residue. This may be due to its physical and chemical properties.

Numerous factors may have contributed to the high occurrence of pesticide residues in the tomato samples in Egypt. Egypt has unwise pest control and uses pesticides heavily to increase crop productivity. Furthermore, most farmers lack the knowledge and sufficient experience in handling pesticides, especially best pesticide practices, the chemical and physical properties of the pesticides, the toxicity of the chemical pesticides and their impacts on human health. In this interim, recent studies have demonstrated that P,P'-DDE and γ -HCH, which are prohibited in Egypt, were found as major pesticide residues in potato tuber samples in Egypt, which is in agreement with this study¹³.

The very high level of pesticide residues leads to various health effects, such as acute effects (headaches, dizziness, eyes and skin problems and nausea); however, there is also strong evidence of long-term effects, such as carcinogenic, neurological and reproductive effects²⁷⁻³⁰.

The most disturbing findings from this study were the possibility of exposure to multiple pesticide residues from the same or different chemical groups and the presence in certain samples of residues of pesticides, such as γ -HCH, P,P'-DDE and heptachlor-epoxide, which are banned for use on tomatoes.

CONCLUSION

In conclusion, the results recommend that a restricted routine monitoring program for pesticide residues is essential for the producers, consumers and authorities of food quality control to prevent, reduce and control pesticide residues and reduce potential health risks. This study has provided promising information on pesticide residue contamination in tomato samples that could help to set appropriate agricultural legislation to protect the impacted environment for the first time in Egypt. A further study on a longer and larger scale should be conducted on certain vegetables to determine the situation of pesticide residues.

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