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Screening of Pesticide Residues in Fresh Vegetables and Fruits by LC-MS/MS and GC-MS/MS

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To determine maximum residue limit of our samples exported from Antalya province of Turkey, we analyzed 3798 samples of fresh vegetables and fruits from 2009-2012 by LC-MS/MS and GC-MS/MS systems for 130 and 16 pesticides, respectively. Additionally, the results were compared based on three different countries' directives. Our findings showed that pesticide residues were detected in 1063 samples (27.98 %). When 1063 samples were analyzed based on different directives, over maximum residue limits were detected in 211 (5.5 %), 118 (3.1 %) and 95 (2.97 %) samples according to the Russian, European Union and Turkish directives, respectively. These samples were mainly tomatoes, cucumbers, bell peppers, lemon and pomegranate. Additionally, acetamiprid, azoxystrobin, carbendazim, imidachlorpid were four of most frequently pesticides detected in our samples when evaluated them based on pesticide type. It is necessary to conduct analysis not only in exported products but also making routine analysis in nationally consumed fruits, vegetables and food products on grocery store shelves.

Keywords: Pesticides, Multi-residue analysis, LC-MS/MS, GC-MS/MS, Fruit, Vegetable.

INTRODUCTION

Pesticides are substances having many different chemical structures including carbamates, organophosphorous compounds, sulphonylureas, triazines[1]. Since 20th century pesticides have been used worldwide in agriculture at various stages of cultivation and also for post-harvest treatment to provide protection against a range of pests[2]. The maximum residue limits (MRL) of pesticides in foodstuffs ought to be determined in many countries because of the crucial risk of pesticides for human health and the quality of agricultural practice[2]. According to the directives of international trade, all consumers have to ensure that foodstuffs contain either up to a certain level or no pesticides and their metabolites at all[3]. So analytical methods are necessary to screen, confirm and quantify as many residues as possible in samples. Analytical methodologies employed must be capable of residue measurement at low levels and must also provide unambiguous evidence to confirm both the identity and the concentration of any residue detected[4]. At this point, multi-residue methods provide the basic tools to the analyst for determining these residues. That is highly preferable due to the simplicity of determining several pesticides in a single extraction, facilitating the demands of more efficient and rapid monitoring[5].

The multi-residue analysis of pesticides in foodstuffs has been carried out by either gas chromatography (GC) or high-performance liquid chromatography (LC) normally coupled with mass spectrometry (MS). First, GC-MS has been developed as the main analytical technique as pesticides had non-polar and volatile properties. Although remarkable benefits of GC-MS/MS, the more useful and selective analytical techniques are needed for the multi-residue analysis of polar and non-volatile pesticides. In recent years, LC-MS/MS considered as an excellent alternative technique for analysis of these compounds. In contrast to GC-MS, LC-MS is very effective in separating non-volatile, thermally labile and high polar compounds[2]. Of the different ionization techniques, electro-spray ionization (ESI) identified as a universal technique has more suitability for analysis of frequently used pesticides. Additionally, the sample pre-treatment steps can be reduced, reliable quantification and confirmation can be easily achieved at the low concentration levels required[6].

The main objective of the present work was to investigate sensitive and reliable screening of 146 pesticides in fresh vegetables and fruits by GC-MS/MS and LC-MS/MS using a rapid, specific and sensitive multi-residue method based on the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) sample preparation method[7]. Also taking into account that a broad

range of pesticides are used to prevent insects, fungi, *etc.* which may result in the presence of pesticide residues on fresh products. This paper reports a really large number of pesticides and metabolites screened in fresh vegetables and fruits by GC-MS/MS and LC-MS/MS, from Turkey. In Turkey as an agricultural country, there is a large production of fresh vegetables and fruits that are both exported and used within Turkey. It is extremely important to screen, confirm and quantify as many residues as possible in samples. Besides, this is the first paper including data concerning the screening of pesticides in 3798 samples of fresh vegetables and fruits of national market between 2009 and 2012. The maximum residue limits values obtained in all samples were evaluated according to all three of the European Union (EU), Turkish and Russian directives.

EXPERIMENTAL

Pesticide standards of analytical grade were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and AccuStandard (New Haven, CT, USA), purity = 98 %. LC/MS/MS mobile phase components (water, methanol, ammonium acetate), acetonitrile, acetic acid, anhydrous magnesium sulphate and sodium acetate were HPLC grade and obtained from Merck (Darmstadt, Germany) and JT Baker (New Jersey, USA). 3798 samples of fresh vegetables and fruits including 2401 tomatoes, 681 cucumbers, 146 pomegranates, 114 bell pepper, 89 lemons, 54 zucchinis, 41 oranges, 38 eggplants, 35 apricots, 34 cherries, 31 strawberries, 26 grapefruits and others were brought to laboratory by responsible personnel of agricultural ministry from 2009 to 2012.

QuEChERS method was used for the sample preparation[7]. Although this methodology was first reported in 2003, it has already been widely accepted by the international community of pesticide residue analysts. Based on QuECHERS method, extraction of the pesticides was performed using acetonitrile.

GC-MS/MS conditions: Gas chromatography analyses were performed using a 3900 Gas chromatograph (Varian Inc., Middelburg, The Nederlands) equipped with a 1079 universal capillary injector and a CP-8400 auto sampler. The injector temperature was held at 275 °C during injection. The column was held at 70 °C for 2 min after injection and then the following temperature program was employed: initial temperature of 70 °C held for 2 min; increased to 150 °C for 5.20 min; increased to 200 °C for 21.87 min; and finally increased to 280 °C for 39.87 min. The injection volume was 2 µL for all standards and samples. A mass-range of *m/z* 50-350 was scanned to confirm the retention times of analytes. Separation was done using a VF-5 FS 30 m × 0.25 mm column from Varian. Helium (99.99 % purity) was the carrier gas at a flow rate of 1.2 mL/min.

Gas chromatography system was coupled with a model Saturn 2200 ion trap mass spectrometer from Varian. The mass spectrometer was operated in an electron ionization mode (EI, 70 eV). The MS/MS detector interface temperature was set at 300 °C and ion source temperature at 220 °C and detector voltage at 1850 V. Multiple reaction monitoring (MRM) conditions were experimentally developed for each individual pesticide on the instrument used in this work. For instrument control, data acquisition and processing Varian MS Workstation, version 6.6 was used.

LC-MS/MS conditions: Waters Alliance 2795 (Milford, MA, USA) complete with a quaternary solvent gradient pump, degasser, column heater module, autosampler, heated column compartment was used for liquid chromatography. Chromatographic separation was performed using a Symmetry C18 (75 × 4.6 mm, 3.5 µm particle size) analytical column (Waters, Milford, MS, USA), with a mobile phase flow rate of 0.5 mL/min and injection volume of 20 µL. Separation was carried out using a gradient between methanol and 5 mM ammonium acetate. The liquid chromatography gradient was operated by the following two eluent components: A: water : methanol (9:1, v/v) containing 5 mM ammonium acetate; B: water : methanol (1:9, v/v) containing 5 mM ammonium acetate. The column was needed to rinse with methanol/water between each experiment. Temperature of the column heater was maintained at 25 °C. Total run-time was 35 min.

Atmospheric pressure ionization-mass spectrometry (API-MS) detections were performed using a Quattro tandem mass spectrometer (Waters, Micromass, Manchester, UK) equipped with an electrospray ionization interface (Z-spray) operating either in the negative or positive mode.

Validation study: Two types of food commodities, which are tomato and lemon as a high water content and high acid content, respectively, were chosen as matrices for the development of a multi-residue method. The accuracy and precision of the method was tested *via* recovery experiments performed by using "blank" fruit and vegetable samples (tomato, lemon) spiked at three concentrations 0.04, 0.02 and 0.2 mg/kg. The limit of quantification (LOQ) was established as the lowest concentration assayed. Mean recoveries and precision (repeatability, expressed as coefficient of variation in %) were determined by analyzing spiked tomato and lemon samples at three spiking levels each (Table-1). Accuracy and precision data were obtained from 18 replicate recovery experiments per commodity and spiking level.

RESULTS AND DISCUSSION

The recoveries were determined from the analyses of 112 selected pesticides in the matrices; lemon and tomato spiked at three concentration levels of 0.04, 0.02 and 0.2 mg/kg. The results shown in Table-1 are mean recoveries obtained from 18 different experiments on all two matrices at each concentration level. As seen, the vast majority of the pesticides gave excellent recoveries in the range of 70-110 % for all levels. Relative standard deviations (RSDs) calculated for each pesticide in all matrices at each level were less than 20 %. QuEChERS methodology using acetonitrile has been widely used for many years because of its ability to separate from water upon the addition of salt without the need to employ a non-polar solvent and amenability with gas chromatography and liquid chromatography applications. This method proved to be rapid and highly effective when applied to the determination and surveillance of a wide range of pesticides in vegetables and fruits, with validation results being highly satisfactory in most cases[8].

TABLE-1
VALIDATION PARAMETERS OF SELECTED PESTICIDES ON TWO MATRICES SPIKED AT THREE CONCENTRATION LEVELS OF 0.04. 0.02 AND 0.2 mg/kg

No.	Pesticides	Matrix											
		Tomato						Lemon					
		0.04 mg/kg		0.02 mg/kg		0.2 mg/kg		0.04 mg/kg		0.02 mg/kg		0.2 mg/kg	
		Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)
1	Abemectin	1.15	4.4	1.000	13.7	1.000	7.6	1.063	8.0	1.046	9.2	1.100	6.7
2	Acetamiprid	0.95	8.4	0.97	10.7	0.94	5	0.96	8.2	1.00	10	0.95	10.9
3	Alachlor	0.93	10.8	1.00	11	0.96	7	0.99	7.0	0.99	12.5	0.96	8.1
4	α-Cypermethrin	0.94	9.3	0.99	9	0.91	7.9	1.00	10.9	0.97	13.7	0.98	12.5
5	Atrazine	0.95	5.7	0.98	10.8	0.97	5.4	0.96	12.5	1.000	13	0.97	11.3
6	Azoxystrobin	0.95	8.6	1.00	9.6	0.96	8.7	0.98	14.2	0.98	10.2	0.97	17.9
7	Benomyl	0.97	9.7	0.98	10	0.95	6.4	1.00	9.4	0.99	12.9	0.99	10.9
8	Bensulfuron-methyl	0.92	11.5	0.99	13.3	0.95	13.3	0.99	15.2	1.00	13.2	1.00	9.0
9	Bentazone	1.10	7.7	1.000	8.9	1.000	5.5	1.00	5.7	1.00	7.8	1.100	7.6
10	Bifentrin	0.96	7.4	0.99	7.3	0.93	5.5	1.00	12.2	1.00	13	1.00	8.6
11	Bromoxynil	0.93	11.4	1.00	10.3	0.98	6.3	0.97	6.4	1.00	9.6	0.97	7.5
12	Bupirimate	0.94	8.4	0.99	10.7	0.91	8.5	1.00	12.4	0.97	15	0.98	11.6
13	Buprofezin	0.96	10.3	1.00	10.9	0.98	7.1	1.00	11.0	1.000	12.5	1.00	9.8
14	Carbaryl	0.96	9.5	1.00	13.9	0.96	6.9	1.00	11.3	1.00	13.4	0.93	10.3
15	Carbendazim	0.97	10.2	1.00	10.4	0.95	6.4	1.00	9.3	1.10	13.7	1.00	11.1
16	Carbofuran	0.95	7.5	0.96	5.9	0.97	5.8	0.98	10.5	1.00	11	0.92	8.0
17	Carboxin	1.00	14.5	1.000	11.5	0.97	8.4	0.91	13.6	0.96	16.3	0.99	9.5
18	Clofentezine	0.97	9.8	0.98	13	0.99	8.1	0.98	12.0	1.00	13	1.00	10.2
19	Carfentrazone-ethyl	1.03	13.7	0.95	15	0.95	10.1	0.98	11.0	1.00	10.4	1.00	11.9
20	Chlorfluazuron	0.97	11.8	1.00	14.1	0.98	7.8	1.00	9.5	1.00	10.2	1.00	12.4
21	Chloridazon	0.98	15.2	1.00	11.6	1.00	9.1	1.10	8.6	1.00	12.4	1.00	8.9
22	Chlorpyrifos	0.93	11.6	0.96	7.5	0.97	7.1	1.02	12.0	1.02	15	1.02	8.6
23	Clefoxydim (Profoxydim)	1.05	10.7	0.98	13.1	0.96	11.7	1.00	10.3	0.96	14.1	0.98	11.7
24	Clodinafop-propargyl	0.98	10.5	1.00	13.4	1.00	11.4	1.02	10.5	1.04	10.8	1.00	13.8
25	Cycloate	0.93	9	0.97	11.3	0.95	7.3	1.04	10.7	1.03	10.6	1.03	12.5
26	Cymoxanil	1.00	10.7	1.00	9.5	1.00	12	0.95	11.3	1.00	14.1	1.00	10.0
27	Cypermetrin	0.94	9.6	0.99	8.8	0.92	7.8	1.00	10.7	1.00	10.9	0.98	12.7
28	Cyproconazole	0.96	9.4	1.04	9.9	0.93	6.4	0.95	10.8	1.00	12.9	0.99	10.9
29	Cyprodinil	0.95	10.3	1.04	12.7	0.96	9.6	0.94	6.8	1.00	12.3	0.96	10.8
30	Diazinon	0.94	8.4	0.99	5.9	0.95	7.5	0.96	11.6	0.96	14.2	0.96	9.5
31	Dichlofluanid	1.01	11.6	1.04	13.9	0.99	7.3	1.01	11.2	1.03	15.1	1.06	10.5
32	Difeconazole	0.90	6.1	0.99	8.7	0.92	5.1	0.98	10.1	1.03	9.4	0.99	8.6
33	Dimethenamid	0.95	11.5	0.98	13.5	0.98	11.5	0.88	5.8	0.94	12.4	0.99	10.6
34	Dimethoate	0.98	6.2	1.03	8.6	0.95	6.6	0.93	9.2	1.00	13.4	1.00	8.0
35	Dimethomorph	0.97	6.4	0.99	9.8	1.00	7.6	0.99	11.0	0.99	12.4	0.96	10.0
36	Diniconazole	1.05	13.1	1.02	11.3	1.01	8.5	1.04	9.5	0.99	12	1.08	4.0
37	Dinocap	0.93	8.1	0.96	11.6	0.94	9.2	1.05	10.6	1.03	13.1	0.98	11.8
38	Diphenamid	0.95	13.1	1.01	11.9	1.01	11.1	0.99	10.6	1.03	10.8	0.95	9.6
39	Epoxiconazole	0.91	11.8	0.98	8.2	0.97	6.8	1.03	13.0	1.03	10	1.00	9.0
40	Ethiofencarb	1.00	10.9	0.98	9.9	0.96	5.4	0.96	13.6	1.00	11	0.96	10.2
41	Ethofenprox	1.01	13	1.08	11	1.03	9.4	0.93	10.0	1.04	16.5	1.07	6.8
42	Ethofumesate	1.00	6.6	0.98	10.7	1.00	8.8	0.97	12.1	1.03	11.7	1.00	10.4
43	Famoxadone	0.95	12	0.96	9.7	0.96	7.4	0.98	12.2	1.00	14.5	0.96	10.9
44	Fenamidone	0.99	15.1	0.99	12.6	0.99	8.7	0.97	12.6	0.98	14.1	1.02	10.8
45	Fenazaquin	1.05	10.7	0.99	12.2	0.99	7.9	1.04	9.6	1.02	10.4	1.04	7.0
46	Fenoxaprop-*p*-ethyl	1.07	9.3	1.09	9.8	1.03	5.8	1.06	8.1	1.03	13.9	1.10	4.3
47	Fenoxycarb	0.98	11.4	1.03	11.7	0.96	5.7	0.98	13.1	1.05	10.6	1.03	12.0
48	Fenpropathrin	0.94	11.7	0.96	12	0.95	8.6	1.04	8.7	1.03	10	0.99	10.1
49	Fenpyroximate	1.03	10.7	1.05	11.1	1.02	7.8	0.94	10.1	1.01	14.5	1.06	5.9
50	Fenthion	0.97	12.2	1.00	12.9	0.98	6.4	0.97	10.8	1.05	15.2	0.98	11.2
51	Fludioxonil	1.04	7	0.93	8	0.97	8	1.04	9.8	1.03	14.3	1.01	11.6
52	Fluazifop-*p*-butyl	1.00	11.6	1.04	10.9	1.07	8.2	1.04	7.2	1.03	13.4	1.09	5.2
53	Fluazinam	1.08	7.3	0.99	11.1	1.06	7.6	0.95	10.8	0.99	9.9	1.12	4.0
54	Furathiocarb	0.94	12.2	0.96	10.7	0.94	6.7	0.96	9.2	1.11	12.8	0.98	10.0
55	Haloxyfop-R-methyl	1.02	11.7	1.08	12.4	0.99	11.2	1.05	8.7	1.12	5.9	1.06	5.1
56	Haloxyfop-etoxy ethyl	1.08	7.3	0.98	11.1	1.06	7.6	0.95	10.8	0.99	9.9	1.11	4.0
57	Hexythiazox	0.95	9.8	0.98	9.6	0.94	7.9	1.03	10.9	1.03	11.4	1.03	6.9

58	Imazalil	0.97	14	1.01	12.3	0.97	9.8	0.96	9.9	1.00	13.8	1.06	9.2
59	Iodosulfuron-methyl	0.97	14.1	1.03	11.7	0.96	12.6	1.02	13.9	1.01	14.4	1.03	12
60	Ioxynil	1.07	15.6	0.96	6.8	0.95	7	1.03	8.7	1.05	6.7	1.07	5.7
61	Isoxaflutole	0.98	12.7	1.01	12.3	0.98	12.9	0.95	9.4	0.99	13	1.00	7.2
62	Kresoxim-methyl	0.98	8.3	1.00	11	0.93	10.7	0.95	8.8	1.02	11.2	0.96	11.8
63	λ-Cyhalothrin	0.94	9.6	1.03	12.8	0.97	8.1	1.00	11.2	1.06	12.6	0.95	10.2
64	Lenacil	0.98	13.5	1.02	12.5	1.00	10.7	0.96	12.2	0.98	11.9	1.00	10.3
65	Lufenuron	1.00	9.3	1.03	11.3	1.02	6.8	1.07	9.6	1.02	13.6	1.06	8.2
66	Malathion	0.96	6.4	1.01	7.1	0.99	7.1	1.02	11	1.06	13.4	0.97	10.8
67	Mefenpyr-diethyl	0.99	10.9	0.99	11.2	0.95	12.9	0.97	10.3	1.02	12.3	1.04	10.4
68	Metalaxyl	0.95	7.6	0.97	6.6	0.97	5.8	0.95	11	1.01	12.7	0.98	11.1
69	Metamitron	0.98	16	1.01	12.6	0.94	11.5	1.00	11.4	0.97	14.6	1.06	8
70	Metolachlor	0.94	7.7	0.94	9.1	0.95	7.8	0.89	9.6	1.04	11.2	0.99	11.3
71	Metribuzin	0.98	9.8	0.99	9.8	0.95	7.8	0.97	9.9	0.99	12.9	0.97	9.1
72	Monolinuron	1.00	8.9	1.02	9.5	0.98	5.8	1.01	9.9	1.02	13.8	0.95	10.4
73	Oxadixyl	0.92	7.5	0.97	10.1	0.95	5.9	1.00	10.9	1.01	12.5	0.98	9.5
74	Quizalofop-*p*-ethyl	1.04	13.7	1.05	11.7	1.03	10.4	1.04	10.2	1.07	9.9	1.06	5.6
75	Penconazole	0.96	12.2	1.02	10.7	0.97	9.2	0.95	11.5	1.04	14.4	0.97	14.7
76	Pendimethalin	0.94	7.1	0.97	7.6	0.94	5	1.03	10	1.05	9.1	0.99	10
77	Permethrin	0.95	5.9	1.00	6.1	0.95	6.4	1.04	9	1.01	13.2	0.99	8.5
78	Phosalone	0.95	7	0.97	9.9	0.98	7.6	1.00	8.8	1.03	12.4	0.99	7.5
79	Phenthoate	0.93	6.9	1.00	8.6	0.95	5.9	0.92	13.9	0.97	14	0.98	10.7
80	Phosmet	0.97	4.5	0.99	5.3	0.97	6	0.95	12.8	1.01	10.5	0.96	9.7
81	Pirimicarb	0.99	7.5	1.02	8.5	0.99	5.6	0.98	12.4	1.08	8.1	0.94	10.8
82	Pirimiphos-methyl	0.88	7	0.98	9.3	0.94	7.5	0.93	7.2	1.01	12.9	0.96	10.8
83	Prochloraz	0.84	14.8	0.90	15.1	1.10	14.7	0.82	21.1	1.00	14	1.11	10
84	Profenefos	0.88	5.6	0.96	8.3	0.93	8.5	0.98	11.3	1.05	11.7	1.00	8.5
85	Prometryne	0.94	12	0.94	11.8	1.00	9	0.90	6.9	0.98	9.8	0.99	8.3
86	Propargite	0.96	9.6	0.97	9	0.95	5.9	1.02	10.8	1.05	11.3	0.99	8.2
87	Propaquizafop	1.06	10.1	1.01	11.2	1.01	5.5	1.01	7.7	1.04	12.2	1.08	5.4
88	Propazine	0.98	10.5	1.00	14.5	0.99	8.8	0.94	11.8	1.05	12.9	0.97	8.5
89	Propiconazole	0.97	8.3	1.01	9	0.97	7	1.00	11	0.99	11.5	0.99	6.8
90	Propyzamide	0.97	8.7	1.04	9.3	0.96	6.7	0.96	10.6	1.13	13.1	0.97	13.5
91	Pyrazophos	0.92	8.6	0.95	11.9	0.96	10	1.05	10.5	1.02	14.7	1.05	7.8
92	Pyridaben	0.91	5.3	0.89	7.8	1.01	6.7	0.96	10.6	1.00	9.9	0.98	9.9
93	Pyridaphenthion	0.91	5.3	0.98	8.6	0.95	7.1	0.96	10.4	0.97	13.5	0.96	11.4
94	Pyridate	0.97	15.2	1.04	10.8	1.03	9.7	0.91	11.3	1.02	15	1.04	7.5
95	Pyriproxyfen	0.95	10.6	0.99	9	0.93	9.7	1.03	10.8	1.07	9	1.01	7.7
96	Sethoxydim	1.04	14.9	1.04	9.4	1.01	8.5	1.05	9.4	0.99	12.2	1.09	4
97	Spiroxamine	1.08	9.1	1.00	13.4	1.00	9.3	1.03	11.3	1.05	10.6	1.08	5.5
98	Tebuconazole	0.92	7.9	1.03	10.7	0.99	10	0.98	10.6	1.01	13.3	0.98	12.8
99	Terbuthylazine	0.92	10.1	0.98	12.2	0.98	11.7	0.91	9.6	0.97	13.6	0.99	12
100	Terbutryn	0.93	7.4	0.99	6.9	0.95	6.5	0.96	11.2	1.00	16.4	0.99	14.5
101	Thiacloprid	0.99	12.9	1.04	14.6	0.99	10.6	1.02	11.1	0.99	14.7	1.05	9.9
102	Thiabendazole	0.96	11.4	0.99	14.2	0.95	10.3	0.96	10.6	0.96	15.6	1.06	10.3
103	Thiophonate-methyl	0.95	10.2	1.01	11.6	0.94	5.6	0.95	11.6	1.07	12.2	0.99	10.7
104	Tolyfluanid	0.97	9.4	0.99	9.3	0.99	6.5	1.02	12	0.97	13.9	0.95	17.1
105	Tralkoxydim	0.96	13.8	1.08	12.3	1.01	11.4	0.99	14.8	0.97	9.8	0.96	12.2
106	Triadimefon	0.97	7.6	1.03	6.6	0.94	7	0.98	12.5	0.97	14.3	1.02	12.6
107	Triadimenol	1.01	11.4	1.01	12.1	0.94	6.8	1.03	10.8	1.07	17.1	1.04	7.9
108	Triallate	0.94	7.9	0.99	12.4	0.92	4.8	1.03	10.7	1.03	14.9	0.97	9.3
109	Trichlorfon	0.97	8.4	1.01	12.5	0.94	6.2	0.96	11.6	1.07	13.3	0.98	9.6
110	Trifloxystrobin	0.94	8	0.98	9	0.97	8.3	0.92	11.2	0.98	12.3	0.99	8.2
111	Triflumizole	0.95	8	1.04	9	0.98	8.3	1.02	11.2	1.04	12.3	1.03	8.2
112	Triticonazole	0.96	11	0.96	13.1	1.00	13.4	0.94	12.7	0.98	15.1	1.04	9.6

3798 samples of fresh vegetables and fruits including 2401 tomatoes, 681 cucumbers, 146 pomegranates, 114 bell pepper, 89 lemons, 54 zucchinis, 41 oranges, 38 eggplants, 35 apricots, 34 cherries, 31 strawberries, 26 grapefruits and others were sent to laboratory by ministry of agriculture from 2009 and 2012 and analyzed in routine for 130 and 16 pesticides by LC-MS/MS and GC-MS/MS system, respectively, based on the polarity or volatility of substances. There is a big potential of export to Russia in Antalya, which is considered to be a city of agriculture.

Pesticides were selected primarily based on Turkish-Russian study groups' agreement. We also included the pesticides most commonly used in Turkey. According to Turkish and Russian regulations, all samples exported to Russia are analyzed by Turkey's private and governmental laboratories and the samples including pesticides over maximum residue limits are not allowed to be exported.

Of the 3798 samples analyzed using both LC- and GC-MS/MS systems, pesticide residues were detected in 1063 (27.98 %). Among these samples, bell pepper, tomato, cucumber,

pomegranate and lemon were five of most frequently analyzed species for pesticides as 70 (61.4 %), 612 (57.5 %), 226 (21.2 %), 28 (19.2 %) and 15 (16.8 %), respectively. Table-2 summarizes the results obtained for samples analyzed between 2009 and 2012 with the following criteria: species, containing residue < maximum residue limits, containing residue > maximum residue limits or not at all. When we analyzed 1063 samples containing pesticide residues based on different directives; over maximum residue limits were detected in 211 (5.5 %), 118 (3.1 %) and 95 (2.97 %) samples according to the Russian, European Union and Turkish directives, respectively. Russian codex has the lowest maximum residue limit levels for pesticides when compared to others. On the other hand, the maximum residue limit levels of European Union and Turkish directives have similarity. According to Environmental Protection Agency, 90 % of fungicides, 60 % of herbicides and 30 % of insecticides are known to cause cancer such as liver[9], breast[10] and prostate[11]. Laboratory studies also indicate that pesticides can cause other serious health problems including infertility, nerve damages, learning disorders, aggressive behaviour[12]. In addition to the laboratory studies, studies of farm workers suggest a link between pesticide usage and cancers such as brain, stomach, prostate, multiple myeloma leukemia, lymphoma and Parkinson's disease[15]. It has been reported that all individuals, but especially children are, sensitive to damages occurred by pesticides[13]. We have to remember that allowed levels do not mean safe levels where our children are concerned. The results are especially important for children, who are more sensitive to pesticides than adults. Pesticide could contribute to learning disorders in children[14]. In Turkey the government and private laboratories are continually improving their approaches toward pesticide analysis to protect human health from exposure to pesticide residues. The regulations should also help enforcing routine analysis in national market.

It is important that one should eat reasonable amounts of fresh vegetables and fruits because of cumulative amounts of pesticide residues. At this point, acceptable daily intake (ADI), which means the amount measured in mg of pesticide per kg bodyweight, is a important safety level for pesticide residues. We detected the pesticide levels in all samples as a ppb (parts per billion) unit. It means that our pesticide levels detected in

TABLE-2
DISTRIBUTION OF 3798 SAMPLES OF FRESH VEGETABLES AND FRUITS ANALYZED BETWEEN 2009-2012 BASED ON EUROPEAN UNION. RUSSIAN AND TURKISH DIRECTIVES

Fruits/ Vegetables	European Union			Russian			Turkish			Total sample number
	Without residue	Residue< MRL	Residue> MRL	Without residue	Residue< MRL	Residue> MRL	Without residue	Residue< MRL	Residue> MRL	
Pear	4	1	-	4	1	-	4	1	-	5
Avocado	-	3	1	-	3	1		4		4
Quince	2	-	-	2	-	-	2	-	-	2
Bell Pepper	44	62	8	44	57	13	44	64	6	114
Wheat	2	6	1	2	4	3	2	7	-	9
Strawberry	26	4	1	26	3	2	26	5	-	31
Tomato	1789	554	58	1789	520	92	1789	562	50	2401
Apple	1	8	-	1	6	2	1	8	-	9
Grapefruit	23	3	-	23	3	-	23	3	-	26
Carrot	-	-	1	-	-	1	-	-	1	1
Persimmon	1	-	-	1	-	-	1	-	-	1
Spinach	1	-	-	1	-	-	1	-	-	1
Fig	3	2	-	3	2	-	3	2	-	5
Zucchini	42	12	-	42	12	-	42	12	-	54
Cauliflower	2	-	-	2	-	-	2	-	-	2
Watermelon	2	4	-	2	4	-	2	4	-	6
Melon	2	4	1	2	4	1	2	4	1	7
Apricot	28	5	2	28	5	2	28	5	2	35
Celery	2	-	-	2	-	-	2	-	-	2
Cherry	27	5	2	27	5	2	27	7	-	34
Kiwi	3	-	-	3	-	-	3	-	-	3
Cabbage	1	-	-	1	-	-	1	-	-	1
Lemon	74	11	4	74	9	6	74	13	2	89
Mandarin	9	-	-	9	-	-	9	-	-	9
Parsley	1	-	-	1	-	-	1	-	-	1
Banana	1	-	-	1	-	-	1	-	-	1
Pomegranate	118	23	5	118	23	5	118	23	5	146
Potato	-	-	1	-	-	1	-	-	1	1
Eggplant	20	18		20	14	4	20	18	-	38
Leek	1	-	-	1	-	-	1	-	-	1
Orange	31	8	2	31	7	3	31	10	-	41
Cucumber	455	195	31	455	160	66	455	199	27	681
Peach	18	2	-	18	-	2	18	2	-	20
Grape	2	13	-	2	9	4	2	13	-	15
Sour cherry	-	2	-	-	1	1	-	2	-	2
Total	2735	945	118 (%3.1)	2735	852	211 (%5.5)	2735	968	95 (%2.97)	3798

fresh products were given not for one product but for one kg of the product. These levels were determined by the government depending on each population's needs. Therefore, we did not additionally need to calculate the acceptable daily intake levels. maximum residue limits already give us the required safety levels, since they are set to ensure consumption of pesticides. There is also a formulation between acceptable daily intake and maximum residue limit as described as maximum residue limit = ADI × W (body weight, kg). When we evaluated the samples with residue levels over maximum residue limits, the levels were obtained in 92 (3.8 %), 58 (2.4 %) and 50 (2 %) of 2401 tomato samples, in 66 (9.7 %), 31 (4.5 %) and 27 (3.96) of 681 cucumber samples, in 13 (11.4 %), 8 (7 %) and 6 (5.2 %) of 114 bell pepper samples and in 6 (6.7 %), 4 (4.5 %) and 2 (2.2 %) of 89 lemon samples according to the Russian, European Union and Turkish directives, respectively. Additionally, of 146 pomegranate samples, over maximum residue limit residues were detected in 5 (3.4 %) according to all three directives. Among these five species, bell pepper had the ratio including most pesticide residue. This result is in concordance with the report of Environmental Working Group. According to the "Dirty Dozen List" defined by this group, bell pepper is one of the 12 most contaminated fresh food products in United States[16]. The other members of the list are peaches, apples, carrots, celery, cherries, grapes, kale, lettuce, nectarines, pears and strawberries. As seen, most of them are fruit samples. Therefore, we should take into account that tomato and cucumber, which account for 81 % of our group, are the most widely grown products in Antalya, are not on the top of the list of most contaminated fresh products. We obtained in general 5 % over maximum residue limits, but we should also keep in mind that these samples were the ones to be exported to Russia mainly and were mostly vegetables; 3306 of 3798 samples (87 %) were vegetables.

In addition to the analysis of samples obtaining pesticide residues based on species, we evaluated the pesticide types. 146 pesticides analyzed in 3798 samples distributed as insecticide (42), herbicide (43), fungicide (45), acaricide (14) and nematicide (2). Most of the samples contained multiple residues. Of the 146 pesticides, acetamiprid, azoxystrobin, carbendazim, imidacloprid were four of the most frequently detected pesticides in our sample groups and the detected sample numbers were 209, 109, 109 and 71, respectively. When evaluated them based on pesticide source, insecticides and fungicides were found in large amounts. Among these, carbendazim is one of the banned pesticides according to the Russian directive. In addition to carbendazim, carbaryl, dimethoate, dichlorfluanide, benamyl and pyrazophos are the members of same banned pesticide list above. These banned pesticides were obtained in 7, 1, 9, 5 and 2 samples, respectively. Carbamates are known to be extremely toxic[17]. Overexposure of individuals involved in the production, transportation and end use of these carbamates can result in serious adverse health effects. Similarly, carbamates elicit the sign of acute intoxication by virtue of the reversible inhibition (carbamylation) of AChE at the synapses and neuromuscular junctions[18]. Carbamates led to a significant reduction in the level of testosterone hormone and significant increase in the level of FSH, LH and prolactin. Kackar *et al*. found that chronic exposure to mancozeb, which is a carbamate pesticide, in male rats (30 days) caused a significant testicular dysfunction as indicated by a marked reduction in serum testosterone level and sperm count[19].

Additionally, benomyl is a carbendazim-derivative. Its primary effects are on the testis. Sloughing is caused by the effects of the chemical on microtubules and intermediate filaments of the Sertoli cell. These effects spread to dividing germ cells and also lead to abnormal development of the head of elongating spermatids[20].

Acetamiprid, which is the most detected pesticide type in our samples, belongs to a new class of insecticides called neonicotinoids. Neonicotinoids act as selective agonists at the nicotinic acetylcholine receptors. This insecticide has been released to the market only within the last decade. That's why the cases of acetamiprid poisoning are not common. However, a case of acute acetamiprid poisoning, who had the blood concentration of acetamiprid, has been reported recently[21].

To our best of knowledge, this study analyzing 146 pesticide residues using LC- and GC-MS/MS in 3798 samples is the first report to have published results in Turkey. Therefore, the data obtained in this work is very important for our country, which has a high potential for export. The exporters which formed the study group knew for sure that their products would be analyzed and would not be allowed to be exported if they exceeded the MRLs. Yet, 5.5 % residue was detected. Around the world, there are violations of maximum residue limits and governments are taking action to improve the situation. There is a prompt need in Turkey to initiate progress to reduce pesticide usage in agriculture and enforce pesticide analysis in national market. All the above findings remind us once more that we have a right to know what is in what we eat.

Conclusion

3798 Samples of fresh vegetables and fruits, which are 2401 tomatoes, 681 cucumbers, 146 pomegranates, 114 bell pepper, 89 lemons, 54 zucchinis, 41 oranges, 38 eggplants, 35 apricots, 34 cherries, 31 strawberries, 26 grapefruits and others, were analyzed for 130 and 16 pesticides by LC-MS/MS and GC-MS/MS system, respectively, between 2009 and 2012 from Turkish market. Of 3798 samples, 211 (5.5 %), 118 (3.1 %) and 95 (2.97 %) samples were obtained to have pesticide residues with over maximum residue limits according to the Russian, European Union and Turkish directives, respectively. Given the importance of exportation potential of Antalya region of Turkey, our pesticide residue analyses in such a large number of samples help us to understand where we are and also to guide the further requirements in terms of reliability and control of exported samples from our region.

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