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Multi-residues Analysis of Pesticides using Gas Chromatography Mass Spectrometry: 1- Leafy Vegetables

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ABSTRACT

The aim of this study is to monitor the pesticide residues in leafy vegetable and to develop the efficient Liquid Chromatography for the extraction and GC/MS for the analysis of investigated 86 pesticides in leafy vegetables. More than 550 samples of leafy vegetables have been collected from Riyadh, Al-Tamer vegetables market, one of the three major fruit and vegetable markets in Riyadh, Saudi Arabia during two years, i.e., 2007-2008. A multi-residue analysis method was developed and described for simultaneously determination of 86 pesticides commonly used in crops, usmg a broad range of physico-chemical properties in leafy vegetables related to Organophosphorous, Organochlorines, Pyrethroids and Carbamates which commonly used in agriculture commodities. Good sensitivity and selectivity of the method were obtained with the limits of quantification 0.0001 mg kg^{-1} in almost of all. The method was satisfactorily applied to routine analysis as a complement to traditional GC-MS method and the limit of detection was generally 10-20 times lesser than the Maximum Residue Levels (MRLs) established by Codex Alimentarius Commission. Pesticide residues were detected in 24.69% of the tested samples (140 samples from the total of 567 samples). Meanwhile, the detected pesticides concentration had been exceeded the MRL in 18.34% of the total tested samples under this investigation.

Key words: Leafy vegetables, pesticides, residue analysis, GC-MS

INTRODUCTION

The human health protection from exposure to pesticides residues in food stuffs remains a major objective in kingdom of Saudi Arabia. Pesticides constitute a very important group of chemical compounds that have to be controlled due to their higher toxicity and widespread use agricultural practice for field and post-harvest protection. Pesticides wide use could lead to extensive pollution of the environment and constitutes a potential and/or deliberate risk to human health because some of these pesticide classified as a probable human carcinogen (El-Saeid, 1999; Eskenazi *et al.,* 1999, 2004; Clegg and Van Gernert, 1999; Brocket *al.,* 2000).

Indeed over 1000 compounds may be applied to agricultural crops in order to control undesirable moulds, insects or weeds. To ensure the safety of food for consumers, numerous legislations such as codex directives (CODEX Committee on Pesticide Residues, 2003) have established maximum residue limits (MRLs) for pesticides in foodstuffs.

Recently, many investigations were reported that the persistence of different pesticides left residual amounts in fruit and vegetables form many areas with different residues levels (EL-Saeid, 2003; Sandra *et al.,* 2003; Fanggui *et al.,* 2006; Medina-Pastor *et al.,* 2008; Galt, 2009; Zorka and Serdar, 2009; Lehotay *et al.,* 2010; Keikotlhaile *et al.,* 2010; EL-Saeid and Haseeb, 2010).

Pesticides residue analysis in agricultural commodities were compared with other organic trace analysis have some peculiarities: (1) a wide range of analyses, with different polarities, solubility at different concentrations levels, may be determined in the same sample; (2) there is a wide range of commodities with different matrix effects in the determination of analysis due to different water and fat content and biochemical composition; (3) Certified Reference Materials (CRMs) are not available (Christer *et al.,* 2004; Pan *et al.,* 2008; Gonzalez-Rodriguez *et al.,* 2008).

Pesticides residues analyses are routinely carried out by means of multi-residue methods based on homogenization of the sample with an appropriate solvent, separation of the liquid portion of the sample from insoluble from material, purification and clean up by florisil column followed by final chromatographic determination step. Organic solvents commonly used to extract pesticides residues in fresh fruit and vegetables are acetonitrile, Petroleum Ether (PE) and diethyl ether (AOAC, 2000). An extensive clean-up of organic extraction is necessary to reduce adverse effects related to the quantification of residues such as the masking of residue peaks by co-eluted matrix component, the occurrence of false positives and/or the inaccurate quantitation. In this studying, we developed more efficient LLC for the extraction and GCMS method for the analysis of 86 pesticides in more than 500 samples of leafy vegetables collected from the one of the three biggest and major fruit and vegetables markets in Riyadh. These pesticides were detected by gas chromatography mass spectrometry (GC/MS) in the electronic ionization mode (EI).

MATERIALS AND METHODS

Reagents and equipments: All pesticides standers were obtained from (Riedel de Haen, Germany and Supelco, USA). We prepared 1 mg mL _,stock solution of each by dissolving 20 mg of the pure analytical standard in 20 mg of acetone. A single composite standard solution was prepared by diluting with acetone according to the Limit of Quantification (LOQ). All standard solutions were stored in glass-Stoppard flasks at 4°C mixed compound calibration solution were prepared in acetone and they were used as spiking solution. Solvents (residue analysis grade) used were acetone, acetonitrile, petroleum ether and other reagents such as sodium chloride and anhydrous sodium sulphate, florisil60-100 mesh for residue analysis were also purchased from (Fluka). The florisil and anhydrous sodium sulphate were activated at 100°C over night and stored in 500 mL glass flaska with glass stoppers and stored in oven at 100°C. The equipments used included a high-speed blender with a stainless steel jar (waring, USA), a shaking separation final (GFL, Germany), a rotavapor, R 215 and cooler circulator chiler B-740 (Buchi, Switzerland), Buchner funnel and chromatographic tubes with Teflon stopcocks and course fritted glass (Agilent, USA) and syringes (Hamilton Bonadus AG, Switzerland). All glassware were rinsed thoroughly using soap and deionization water, then washed with acetone and dried in oven (100-130°C) over night.

Samples collection: This studying was conducted during two years from January 2007 to December 2008. Five hundred sixty seven leafy vegetables samples (rocket, lettuce, coriander, corchorus, cabbage, parsley, basil, spinach, radishes dill, mint, green onion, chard and leek) have been collected from Al-tamer vegetables market in Riyadh which is a wholesale market is collecting

the leafy vegetables from more than 250 farms allover Saudi Arabia. Samples were put in sterile polythene bags and transported to the laboratory where they were analyzed immediately or stored at 4°C until analysis within 24 h.

Extraction and partitioning method: The chopped leafy vegetable samples (100 g) were placed in a stainless steel jar 1 Land extracted with 200 mL of acetonitrile and 10 g celite, the blender was vigorously homogenized into high speed for 2 min the mixture was filtrated by using Buchner funnel fitted with shark-skin filter paper into 500 mL suction flask. An aliquot of organic was transferred to 1 L separator funnel and added 100 mL of (PE), the mixture was vigorously shaken for 1-2 min and then was added 100 mL saturated solution of NaCl and 600 water. The mixture was vigorously mixed and the separator funnel was allowed to be held horizontal position for few minutes. The aqueous layer was discarded and the solvent layer was washed with twice time 100 mL portions of distilled water and the washed layer were transferred into 100 mL beaker and washed with 15 g of anhydrous sodium sulphate. Finally, the extract was concentrated to 5 mL volume and transferred directly to florisil column.

Extract cleanup: Florisil column cleanup was conducted according to the AOAC (2000). The column was prepared containing about 12 em activated florisil topped with 1 em anhydrous sodium sulphate, column was washed by 40 mL (PE) and than was added extract concentrated to 5 mL and was allowed to pass through the column. The walls of the tube were rinsed additional small portions of petroleum ether and elute at 5 mL min_, with 200 mL 6% eluting solvent (Diethyl ether in (PE)) and then 200 mL 15% and finally 200 mL 50% eluting solvent (Diethyl ether in (PE)) at 5 mL min^{-1}

Determination method: Chromatographic instrumentation and quantification were carried out by Gas chromatograph-mass spectrometer GC-MS (Aglient model 6890N) coupled with (model 5975B) quadrupole mass spectrometer (Masahiro *et al.,* 2005) with a GC column HP-5MS 5% phenyl-95% methyl siloxane, 30x0.25 mm id x 0.25 f!m film thickness. The GC operating conditions: splitless injection, injector temperature 250°C, helium carrier gas (99.9999 purity) at flow rate 0.9 mL min^{-1} with column head pressure 7.4 psi, oven temperature from 70°C (2 min hold), then raised to 130°C at the rate (25°C min^{-1}) afterwards raised to 220°C at (2°C min^{-1}) and then raised to 280°C at (10°C min^{-1}) and eventually (4.6 min hold). The sample (1 f!L) was injected in splitless modes. The MS system was routinely set in selective ion monitoring (SIM) mode and each compound was quantiaited based on peak area using one target and one or two qualifier ion. Mass spectrometer parameter was set as follows: electron impact ionization mode with 70 eV electron energy, scan mass range 100-400 at 0.62 sec/cycle. Ion source temperature 230°C, MS quad temperature 150°C, EM voltage 1450 and solvent delay 4 min. Table 1 summarizes the parameters of retention time, LOQ, Target and qualifier ions m/z by scan mode of pesticides were studied in leafy vegetable samples.

RESULTS AND DISCUSSION

A multiresidue procedure was carried out to monitor the pesticide residues in a wide range of the most common consumed leafy vegetables samples collected during two years, i.e., 2007-2008. The analyzed samples composed of fourteen species of leafy vegetables, i.e,. rocket, lettuce, coriander, corchorus, cabbage, parsley, basil, spinach, radishes, dill, mint, green onion, chard and

Table 1: Parameter of retention time, LOQ, target and qualifier ions m/z by scan mode

					Qualifier ions (m z $^{-1}$)	
n	Compounds	Retention time (min)	LOQ (μg kg^{-1})	Target ion (m z-^{1})	Q,	
1	Dichlorvos	7.211	0.02	109	185	79
2	Propamocarb	9.849	0.04	58	71	129
3	Mevinphos	10.828	0.09	127	192	109
4	Chloroneb	13.015	0.01	191	193	206
5	Methomyl	14.837	0.10	105	88	57
6	Propachlor	16.35	0.02	120	77	176
7	Propoxur	16.44	0.03	110	152	81
8	Ethoprophos	17.183	0.05	157	97	139
9	Bendiocarb	18.808	0.01	151	126	166
10	Sulfotep	19.35	0.01	322	202	97
11	Alfa-BHC	19.449	0.02	183	181	219
12	hexachlorobenzen	19.949	0.05	284	249	142
13	Diehlor an	20.412	0.03	176	206	124
14	Dimethoate	20.694	0.02	87	93	125
15	Simazine	21.24	0.01	201	186	173.2
16	Carbofuran	21.517	0.01	164	149	123
17	Lindan	21.953	0.01	219	181	111
18	Fonofos	22.837	0.06	109	137	246
19	Delta-BHC	23.934	0.05	181	219	111
20	Diazinon	24.181	0.03	179	137	152
21	lprobenfos	25.472	0.04	91	204	122
22	Pirimicarb	26.194	0.05	166	72	238
23	Dichlorlenthion	26.869	0.01	279	223	162
24	Phosphamidon I	27.074	0.03	127	72	264
25	Phosphamidon II	27.105	0.05	127	72	264
26	Chlorpyrifos-Me	27.53	0.03	286	125	288
27	Vinclozolin	27.643	0.02	212	285	187
28	Carbaryl	27.846	0.03	144	115	116
29	Alachlor	28.292	0.04	160	188	146
30	Ronnal	28.738	0.02	285	287	125
31	Metalaxyl	28.894	0.05	206	146	192.2
32	Fenitrothion	30.004	0.07	277	125	109
33	Linuron	30.118	0.04	61	187	124
34	Aldrin	30.417	0.02	66	263	91
35	Thiobencarb	30.794	0.08	100	72	125
36	Malathion	31.31	0.03	127	173	99
37	Fenthion	31.679	0.03	278	125	109
38	Pirimiphos-ethyl	34.279	0.05	318	333	304
39	Capten	34.791	0.06	79	151	114
40	Chlorofenvenphos	35.549	0.02	267	323	269
41	Chlordan-trans	35.99	0.04	373	375	237
42	Alfa-endosulfan	36.919	0.09	239	237	195
43	Nanchlor-trans	37.168	0.01	409	100	237
44	Chlordane-cis	37.311	0.05	375	373	377
45	Disulfoton sulfon	37.67	0.03	213	153	97
46	Dieldrin	39.172	0.02	79	265	81

Table 1: Continued

n	Compounds	Retention time (min)	LOQ (μg kg^{-1})	Target ion (m z^{-1})	Qualifier ions (m z^{-1})	
47	P,P-DDE	39.688	0.01	246	318	248
48	O,P-DDD	40.321	0.01	235	237	165
49	Endrin	40.92	0.02	263	265	281
50	Beta-endosulfan	41.82	0.03	207	239	195
51	Chlorobenzilate	42.683	0.04	251	139	253
52	P,P-DDD	43.234	0.01	235	237	165
53	Benodanil	43.773	0.03	231	323	203
54	Ethion	44.131	0.01	231	97	153
55	Carbophenothion	45.558	0.04	157	121	125
56	Resmethrin I	48.965	0.03	123	171	143
57	Resmethrin II	49.532	0.03	123	171	143
58	Hexabromobenzen	49.772	0.04	551	554	549
59	Phosmet	50.3	0.03	160	161	77
60	EPN	50.726	0.04	157	169	185
61	Dicofol	50.955	0.02	139	111	251
62	Fenoxycarb	51.04	0.07	255	186	116
63	Tetramethrin II	51.322	0.02	164	123	81
64	Tetradefon	52.029	0.05	159	111	229
65	Mirex	52.46	0.01	272	274	270
66	Furathiocarb	52.62	0.04	163	57	164
67	Amitraz	53.373	0.03	132	121	147
68	Lamda-cyhalothrin	53.77	0.05	181	197	208
69	Azenophos-ethyl	53.882	0.03	132	160	77
70	allethrin I	54.515	0.04	123	181	81
71	allethrin II	54.523	0.04	123	181	81
72	allethrin III	54.59	0.04	123	181	81
73	Permethrin I	54.891	0.02	183	163	165
74	Permethrin II	55.111	0.02	183	163	165
75	Comaphos	55.165	0.02	263	226	109
76	Cyfluthrin III	55.855	0.05	163	165	226
77	Cyfluthrin	55.997	0.04	163	165	226
78	Cyfluthrin IV	56.103	0.04	163	165	226
79	Cyfluthrin II	56.162	0.04	163	165	226
80	Cypermethrin II	56.284	0.03	163	165	181
81	Cypermethrin IV	56.424	0.03	163	165	181
82	Cypermethrin I	56.522	0.03	163	165	181
83	Cypermethrin III	56.575	0.03	163	165	181
84	Fenvalerate I	57.454	0.05	125	167	281
85	Fenvalerate II	57.716	0.03	125	167	281
86	Deltamethrin	58.44	0.02	253	181	181

leek. A wide range of pesticide residues were detected and quantified in the analyzed samples during the two years of this study. In 2007 it was detected the residues of 24 pesticides while in 2008 it was detected the residues of 27 pesticides.

Pesticide residues in leafy vegetables during 2007: Data in Table 2 shows the amounts of the detected pesticide residues in leafy vegetable samples collected from different locations in Saudi

Table 2: Pesticide residues detected (ppm) in leaf'y vegetable samples in year 2007

	Leafy vegetables														
Pesticide	Rocket	Lettuce	Coriander	Corchorus	Cabbage	Parsley	Basil	Spinach	Radishes	Dill	Mint	Green onion	Chard	Leek	Freq
Lindane	0.005		0.009			0.006					0.011	0.002			5.00
Chlordane	0.009													0.017	2.00
Mirex							0.035				0.003				2.00
Endosulfan	0.014			0.15		*om*		0.019	*om*						5.00
Chloropyrifos	0.006			0.007											2.00
Mevinphos		0.002													1.00
Azinophos		0.007													1.00
Phosmet		oms						0.013			0.17				3.00
Ethoprofos						0.013									1.00
Diazinon						0.1									1.00
Iprobenfos	0.011						0.026				0.032			0.011	4.00
Tetramethrin		0.054													1.00
Permethrin			0.113	0.014		0.01		0.065	0.171	0.062	0.075	0.002		0.003	9.00
Deltamethrin					0.529										1.00
Lambda-cyhalothrin										0.006					1.00
Cyfluthrin	0.85					0.007				0.007					3.00
Allethrin	0.008														l.OO
Fenvalerate												0.065			l.OO
Resmethrin	0.005		0.05							0.059		0.148		0.003	5.00
Bendocarb	*om*														l.OO
Fenoxycarb											0.002				l.OO
Teradifon											0.012				l.OO
Linuron				0.007				0.004							2.00
Chloroneb					0.005										l.OO
Amitraz						0.002									l.OO
No. of Detected compounds	9.00	4.00	3.00	4.00	2.00	7.00	2.00	4.00	3.00	3.00	5.00	5.00	l.OO	4.00	
Total detected (ppm)	0.933	0.081	0.172	0.178	0.534	0.158	0.061	0.101	0.197	0.128	0.132	0.388	0.002	0.034	

Arabia during the year 2007. According to the detected pesticides, it is clear that there are a wide range of compounds which included insecticides (25 compounds), herbicides (three compounds, i.e., linuron, chloroneb and amitraz) and fungicides (one compound, i.e., teradifon). The detected insecticide residues which represent the majority of the detected compounds, it was found that such insecticides could be classified chemically into their major four chemical groups, 1.e., organochlorines, Organophosphorus, pyrethroids and carbamates. The detected organochlorines insecticides, i.e., lindane, chlordane, mirex and endosulfan while the organophosphorus insecticides included seven agents, i.e., chloropyrifos, mevinphos, azinophos-ethyl, phosmet, ethoprophos, diazinon and iprobenfos. Eight Pyrethroids compound, were detected and it included tetramethrin, permethrin, deltamethrin, lambda-cyhalothrin, cyfluthrin, allethrin, fenvalerate and resmethrin. carbamates, only two compounds were detected, i.e., bendiocarb and fenoxycarb. The mentioned pesticides were detected in fourteen leafy vegetables, included rocket, lettuce, coriander, corchorus, cabbage, parsley, basil, spinach, radishes, dill, mint, green onion, chard and leek.

According to the detected pesticides in and/or on the leafy vegetables involved in this study, it was observed that total numbers of the detected compounds were found in rocket followed by parsley, mint, green onion, lettuce, leek, corchorus, spinach, coriander, dill, radishes, cabbage, basil and chard. The total number of the detected compounds in such leafy vegetables was 9, 7, 5, 5, 4, 4, 4, 4, 3, 3, 3, 2, 2 and 1 compound, respectively.

The data tabulated in Table 2 also showed the detected amounts of pesticide residues in leafy vegetables could be ranked in descending order as follows: rocket, cabbage, green onion, radishes,

corchorus, coriander, parsley, mint, dill, spinach, lettuce, basil, leek and chard which represent the lower leafy vegetable contained pesticide residues. In term of figures, the sum of the detected pesticide residues in such leafy vegetables were 0.933, 0.534, 0.338, 0.197, 0.178, 0.172, 0.158, 0.132, 0.128, 0.101, 0.081, 0.061, 0.034 and 0.002 ppm, respectively. From such ranking, it was observed that rocket, cabbage and green onion were the most contaminated leafy vegetables.

The frequency of the detected pesticide residues was calculated in the analyzed leafy vegetables, it was found that the most frequent compounds was permethrin followed by resmethrin and linden, endosulfan, iprobenfos, cyfluthrin and phosmet. In term of figures, the frequencies for these pesticides were 9, 5, 5, 5, 4, 3 and 3, respectively. The other detected compounds were frequented between two and one time. From the presented results it could concludes that rocket, cabbage and green onion were the most contaminated leafy vegetables and the pyrethroids are the most frequented pesticides during the year of 2007.

Pesticide residues in leafy vegetables during 2008: In the agriculture season of 2008, data represented the detected amounts of pesticides residues in leafy vegetables collected during the season of 2008 are shown in Table 3. According to the detected amounts and/or compounds it was

Table 3: Pesticide residues detected (ppm) in leaf'y vegetable samples in year 2008

	Leafy vegetables		Freq												
Pesticide	Rocket	Lettuce	Coriander	Corchorus	Cabbage	Parsley	Basil	Spinach	Radishes	Dill	Mint	Green onion	Chard	Leek	Freq
Chloroneb												0.04			1.00
O,P-DDT							0.004								1.00
Endosulfan				0.157			0.113			0.108		0.253	0.131		5.00
Chloropyrifos							O.D13								1.00
Mevinphos	0.007					0.006									2.00
Azinophos			0.001												1.00
Fonofos										0.006					1.00
Comaphos											0.014				1.00
Phosphamidon											0.01				1.00
Ethoprophos						0.014				0.006					2.00
Diazinon			O.D23							0.029					2.00
Pirimiphos-ethyl								0.018							1.00
Dimethoat			0.011				0.125	0.013	0.03		0.051				5.00
tetramethrin												0.001			1.00
Permethrin	0.039														1.00
Deltamethrin					0.029						0.105				2.00
Lambda-cyhalothrin														0.0002	1.00
Cyfluthrin	0.301			0.271				0.25	0.58						4.00
Allethrin		*om*										*om*			2.00
Fenvalerate	0.077														1.00
Cypermethrin	0.168			0.096		0.014									3.00
Resmethrin	*om*					0.033									2.00
Bendocarb			0.005		0.016										2.00
Thiobencarb						0.009									1.00
Carbofuran		0.003													1.00
propamocarb												0.027		0.017	2.00
Benomyl					0.016										1.00
Amitraz	0.002												0.002		2.00
No. of detected compounds			4							4	4				
Total detected (ppm)	0.664	O.D23	0.04	0.524	0.061	0.076	0.273	0.263	0.61	0.149	0.18	0.253	0.131	0.0172	

sobserved that the pattern of the detected pesticide residues in the collected leafy vegetables samples were slightly differed from those presented in 2007 season. For example, in 2008 season, it was found the o,p'-DDT as organochlorine compound, phonophos, comaphos, phosphamidon, pirimiphos-ethyl and dimethoat as organophosphorus, cypermethrin as pyrethroids. Also, it was detected thiobencarb, carbofuran, propamocarb as carbamates insecticides. As for fungicides, it found benomyl only. As for the detected amounts, it is clear that cyfluthrin represented the highest amounts of the detected residues which ranged between 0.25 to 0.58 ppm while the other detected concentrations were ranged between 0.01 to 0.0002 ppm. Overall, the pesticides residues were found in this study were approximate similar to other studies (Dogheim *et al.*, 2004; Amoah *et al.*, 2006; Gonzalez-Rodriguez *et al.*, 2008).

The number of detected residues, in leafy samples could be ranked in descending order as follow rocket, parsley, basil, green onion, coriander, dill, mint, corchorus, cabbage, lettuce, spinach, radishes, chard and leek, the detected number of compound residues was 7.0, 5.0, 5.0, 5.0, 4.0, 4.0, 4.0, 3.0, 3.0, 2.0, 2.0, 2.0, 2.0 and 1, respectively. As for the detected amounts of the mentioned pesticides, the ranking of leafy samples becomes different to be rocket is the most contaminated leafy samples followed by radishes < corchorus <green onion <basil< spinach <mint <dill< chard <parsley< cabbage< coriander< lettuce< leek. In term of figures, the total detected amounts are 0.664, 0.61, 0.524, 0.391, 0.273, 0.263, 0.18, 0.149, 0.133, 0.076, 0.061, 0.04, 0.023 and 0.0172 ppm for the mentioned leafy samples, respectively. In case of the frequencies of pesticide residues between the collected leafy samples, it was observed that dimethoat was the most frequented compound followed by cyfluthrin followed by cypermethrin which their frequencies were 50, 4.0 and 3.0, respectively. The other detected compounds were frequented between two and one time. However, the presented data of 2008 season clearly shows that rocket samples were the most contaminated leafy vegetables followed by radishes followed by corchorus and green onion while both ofleek and chard were the lowest contaminated leafy samples. Data was mentioned previously partially agreement with (Qu *et al.*, 2010; Osman *et al.*, 2010).

The detected amounts of pesticide residues during the two mentioned seasons, i.e., 2007/20008 were compared with that MRL values, it could calculated the average of percentage of leafy vegetables samples contained amounts of residues exceeds the MRL values as shown in Table 4. From such data, it is clear that the majority of the analyzed leafy samples collected in 2007 contained exhibited higher values than those of 2008 except seven leafy vegetables, i.e., cabbage,

Table 4: Average of the percentage oflea:fy vegetable samples exceeding the MRL of pesticide residues during 4 seasons in years 2007-2008

		Leafy vegetables samples exceeding the MRL														
Season	Ye	Rocket	Lettuce	Coriander	Corchorus	Cabbage	Parsley	Basil	Spinach	Radishes	Dill	Mint	Green onion	Chard	Leek	Me=
Winter	2007	33.33	33.33	50.00	25.00	0.00	50.00	0.00	0.00	0.00	50.00	66.67	50.00	0.00	0.00	25.60
Spring		42.86	12.50	20.00	16.67	0.00	16.67	0.00	33.33	0.00	0.00	0.00	33.33	0.00	0.00	12.53
Sumer		33.33	**11.11**	0.00	30.00	0.00	33.33	50.00	0.00	0.00	0.00	30.00	10.00	50.00	50.00	21.27
Autumn		28.57	0.00	40.00	0.00	33.33	20.00	0.00	0.00	14.29	16.67	20.00	9.09	0.00	0.00	13.00
Me=		34.52	14.24	27.50	17.92	8.33	30.00	12.50	8.33	3.57	16.67	29.17	25.61	12.50	12.50	
Winter	2008	25.00	5.56	16.67	33.33	14.29	18.18	33.33	33.33	10.00	33.33	0.00	28.57	0.00	0.00	17.97
Spring		50.00	25.00	40.00	0.00	0.00	33.33	33.33	0.00	0.00	33.33	60.00	50.00	33.33	33.33	27.98
Sumer		20.00	0.00	33.33	0.00	0.00	16.67	0.00	0.00	25.00	0.00	0.00	33.33	0.00	20.00	10.60
Autumn		0.00	0.00	0.00	20.00	40.00	0.00	50.00	25.00	0.00	0.00	16.67	0.00	25.00	0.00	12.62
Me=		23.75	7.64	22.50	13.33	13.57	17.05	29.17	14.58	8.75	16.67	19.17	27.98	14.58	13.33	

basil, spinach, radishes, green onion, chard and leek which were higher in their values than those of 2007. In addition, when the analyzed samples distributed between the four mainly season of each year, i.e., Winter, Spring, Summer and Autumn (based on the analyzed date), neither correlation nor trend could be observed between the pesticide residues content and the mentioned season. The selected plant foods will not give a for adverse biological effects to take place providing the residues of pesticides are controlled to be kept to a minimum. Pesticides residue monitoring programs should then be implemented to assure the minimum allowable residue levels in plant foods, especially with regards to permethrin, endosulfan, dimethoat (Rial-Otero *et al.,* 2005).

The results of the detected amounts of pesticide residues in the selected leafy vegetables, it is therefore clear that patterns of pesticide use are crop dependent: the predominant use of pesticides in leafy vegetables is mainly to control a wide range of lepidopteran larvae. In addition, the most reasonable explanation for the highly detected pesticide residues in rocket, cabbage and radishes may be due to the intensive use of insecticides and the highly deposited amounted of the applied compounds on the broad leafs of such vegetables. Overall, insecticides found in this study were similar to those found in other studies (Cabras and Conte, 2001; Poulsen and Andersen, 2003; Dogheim *et al.,* 2004; Gebara *et al.,* 2005; Amoah *et al.,* 2006).

CONCLUSION

However, the detected amounts of the mentioned insecticides in these important leafy vegetables, make the necessary to continuing the pesticide residue monitoring programs which must be implemented to assure the minimum allowable residue levels in plant foods. In addition, the obtained results clearly indicate the actual situation of the misuse of insecticides which may affect in turn at long period the consumers health. With the LLC and GCMS multiresidue method, the optimum conditions were met to extract and determined 86 pesticides in more than 550 leafy vegetables samples less time and low detection limit (0.001 ppm). Pesticide residues were detected in 24.69% of the tested samples. Meanwhile, the detected pesticides concentration had been exceeded the MRL in 18.34% of the total tested samples.

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