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MULTI-RESIDUES ANALYSIS OF CHEMICAL PESTICIDES IN IMPORTED AND LOCALLY PRODUCED HONEY IN KINGDOM OF SAUDI ARABIA

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ABSTRACT

Ripe honey samples were collected from different regions of Saudi Arabia during honey flow seasons in 2010-2011, to monitor certain pesticides residues in local and imported honey. The Pesticide residues were determined by gas chromatography with mass selective detector (GC–MSD). A multi-residue method was developed and described for simultaneously determination of 86 pesticides commonly used in crop protection. This method used to determine pesticide residues with a broad range of physico-chemical properties in fresh fruit and vegetables related to Organophosphorous, Organochlorines, Pyrethroids and Carbamates mainly used in agriculture. Pesticide residues above maximum concentrations of pesticide residues (MRLs) were detected in 10 honey samples and represent (11%) of 91 samples. The acaricides; amitraze, coumaphos and endosulfan which use against varroa mite disease (*Varroa destructor* Anderson and Trueman) in honey bee colonies were detected in the honey samples. It concluded that a monitoring program for pesticide residues in honey markets is necessary needed.

Keywords: Pesticides, multi-residue analysis, honey, pesticides, residue analysis, GC-MS.

INTRODUCTION

Honey is natural product consumed by many people around the world. It is should bee be free of any chemical contaminations and safe for consumption to child, old and ill people (Tsipi et. al. 1999). It is being used as a natural sweetener in food manufacturing practices, cosmetics and also as a pharmaceutical in the treatment of various human infections. A food becomes wholesome when it meets appropriate nutritional and safety standards as well as specific quality attributes. While the nutritional and quality aspects of honey are very important, safety of honey is also critical, as it determines the consumer acceptance. However, nowadays bee products are produced in an environment not free of contamination sources (Bogdanov, 2006). The additives and preservatives are not allowed for honey; however, there is a recent concern about the presence of antibiotics and pesticides in certain honey samples (Ozlem 2006).

Pesticides play a beneficial role in agriculture industrial, because they help to combat the variety of pest that destroy crops. Even though small amounts of pesticide residues remain in the food supply, constituting a potential risk for the human health, because of their sub-acute and chronic toxicity (Mukherjee, 2009). The most widely used pesticides are

organophosphorus and carbamates, which have almost completely replaced organochlorine pesticides. The extensive distribution of these groups of pesticides causes bees, that have been fed on contaminated blossom, to transfer pesticide residues into honey and finally to the consumer. The occurrence of organochlorines compounds in the food chain has already been reported in several studies (Cruz *et al*., 2003 and Fernandez *et al*., 1995). The pesticide determination in bee products is necessary to monitor contamination and guarantee consumer health (Fernandez *et al*., 2002).

The presence of pesticide residues in honey has impelled the need for setting up monitoring programs to determine the proper assessment of human exposure to pesticides making possible to take policy decisions in the interest of health hazard. Different national regulations have established maximum concentrations of pesticide residues (MRLs) permitted in honey, but the lack of homogeneity causes problems in International marketing and trade. Up to now, maximum limits of pesticide residues in honey are not included in the Codex Alimentarius (1998). The European Union (EU 1996, 1999) legislation has regulated the MRLs for three acaricides: amitraz, coumaphos, and cyamizole, which are 0.2, 0.1, and 1 mg/kg, respectively (EC, 1990). The U. S. Environmental Protection Agency (FDA, USA 2003) has established MRLs for amitraz (1 mg/kg) Germany, Italy, and Switzerland have set MRLs (Rissatoa *et al*., 2007) for amitraz, bromopropylate, coumaphos, cyamizole, flumetrine, and fluvalinate, which oscillate between 0.01 and 0.1 mg/kg in Germany, between 5 and 500 mg/kg in Switzerland, and are of 10 mg/ kg in Italy.,coumaphos (0.1 mg/kg), and fluvalinate (0.05 mg/kg^{-1}). The study performed in 27 honey samples from India from 1993 to 1995 showed that all samples were contaminated by organophosphorus, mainly DDVP, chlorpyriphos, monocrotophos, dimethoate, and fenitrothion; Carbofuran and carbaryl contaminated 55% of the honey samples (Rathi et. al., 1997). Blasco *et al*., (2003) reported that the honey samples from Portugal and Spain contained mostly organochlorine along with other insecticides. They also added that all honey samples studied were also contaminated with organochlorines, but the amount of residues found was much lower than that of organophosphorus and carbamates.

Many methods have been reported for the determination of pesticides in honey. However, honey samples pose substantial analytical problems, particularly to high percentage of sugar or, in some cases, intensive coloration due to pigments. Most methods used for OCPs are based on liquid-liquid extraction (LLE) (Fernandez *et al*., 2002). Pang *et al*., (2006) developed a multi-residue method for the determination of 450 pesticide residues in honey, fruit juice and wine using double-cartridge solid-phase extraction (SPE), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS-MS). The limit of detection for the method was between 1.0 and 300 ng/kg depending on each pesticide analytic. At the three fortification levels of 2.0–3000 ng/kg, the average recovery rates were between 59 and 123%, among which 413 pesticides (92% of the 450) had recovery rates of 70–120% and 35 pesticides (8% of the 450) had recovery rates of 59–70%. There were 437 pesticides (97% of the 450) with a relative standard deviation below 25%; there were 13

varieties (3% of the 450) between 25.0 and 30.4%. Tsipi *et al*., (1999) developed a new clean up method for the routine multi residue determination of organochlorine pesticide residues in honey. The determination of organochlorine pesticide residues was performed by capillary gas chromatography with electron capture detection. The mean recoveries of 18 organochlorine pesticides were estimated at various concentrations and found very efficient in most cases. Their results indicated that the detection limits were found to be between 0.05 and 0.20 µg/kg. Beatriz- Albero *et al*., (2004) developed an analytical method for the simultaneous determination of 51 pesticides in commercial honeys. The detection limits of the method ranged from 0.1 to 6.1 µg/kg for the different pesticides studied. The developed method is linear over the range assayed, 25−200 µg/L, with determination coefficients of >0.996. The proposed method was applied to the analysis of pesticides in honey samples, and low levels of a few pesticides (dichlofluanid, ethalfluralin, and triallate) were detected in some samples. Mukherjee (2009) applied the method of simple technique of liquid–liquid extraction to screen six samples of honey locally available for pesticides residues. Recoveries ranged from 60% to 90.6% with RSDs from 2% to 10%. Low recoveries were recorded for a and b endosulfan in the range of 60%–71%. The LOQs, varied from 0.05 to 1.0 mg/kg.

The objective of this study was to determine the pesticides residues in honey samples.

Reagents and equipments

All pesticides standard were obtained from Riedel de Haen and Supelcom. 1mg/ml stock solution of each by dissolving 20 mg of the pure analytical standard in 20 mg of acetone was prepared. A single composite standard solution was prepared by diluting with acetone according to limit of detection (LOD). All standard solutions were stored in glass-Stoppard flasks at 4^{o}C. Mixed compound calibration solutions were prepared in acetone and they were used as spiking solution. Solvent used (residue analysis grade) were acetone, petroleum ether, diethyl ether, n-hexane, dichloromethane, ethyl acetate and iso-octane. The solvents were purchased from Merck Company, Germany. Anhydrous sodium sulphate, silica gel and florisil (Merck) were used after heating overnight at 120 °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. All glassware were rinsed thoroughly using soap and deionization water, then washed with acetone and dried in oven (100-130 ^{o}C) over night.

Chromatographic instrumentation and quantification

Gas chromtograph-mass spectrometer (Aglient model 6890N) coupled with quadrupole mass spectrometer (model 5975B) with a GC column HP-5MS 5% phenyl - 95% methyl siloxane, 30m x 0.25mm id x 0.25 µm film thickness was used. GC operating conditions were splitless injection, injector temperature 250 ^{o}C, helium carrier gas (99.99 purity) at flow rate 0.9 ml/min with column head pressure 7.4 psi, oven temperature from 70 ^{o}C (2 min hold), then raised to 130 ^{o}C at the rate (25 ^{o}C/min) afterwards raised to

220 ^{o}C at (2 ^{o}C/min) and then raised to 280 ^{o}C at (10 ^{o}C/min) and eventually (4.6 min hold). The sample (1µL) 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 ^{o}C, MS quad temperature 150 ^{o}C, EM voltage 1450 and solvent delay 4 in.

Pesticide Measurements

Eighty-Six pesticides were analyzed and tested throughout the present study in which some of these pesticides are extensively used for controlling pests attacking vegetables and fruits (fig. 1). GC-Ms calibration of the monitored pesticides was done according to the above mentioned methods. Parameter of retention time, peak of area, target and qualifier ions m/z by scan mode were tabulated in Table (1 and 2).

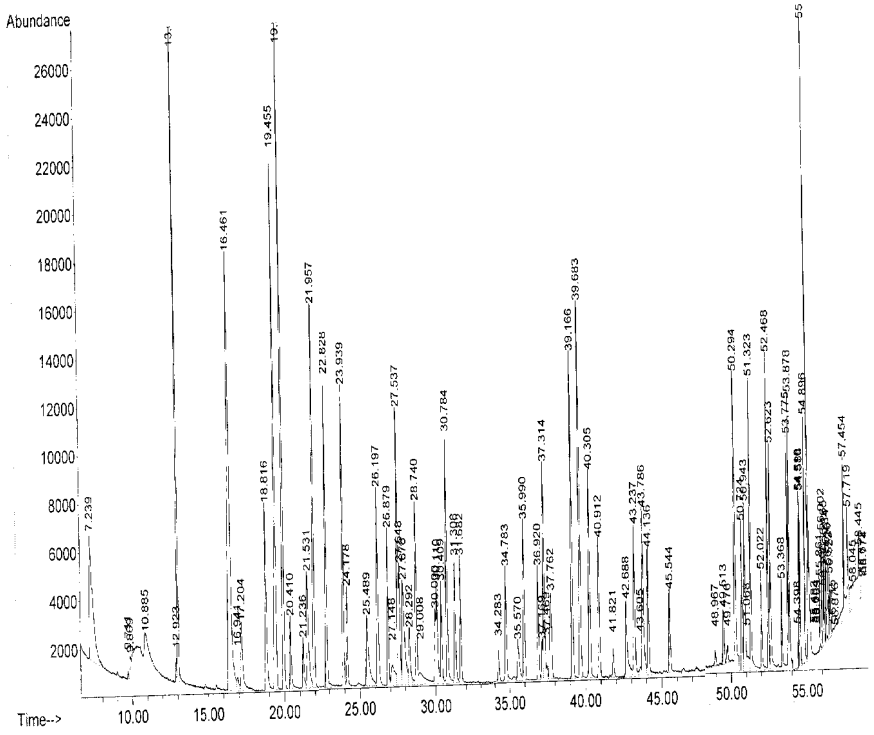

Fig. (1): GC-MS Chromatogram for stander solution of (86) pesticides from (0.1-0.5 µg/ml).

Table (1): Parameter of retention time, peak of area, Target and qualifier ions m/z by scan mode

N	Compounds	Retention time/min	Peak of area	Target ion m/z	Qualifier ions m/z	
					Q1	Q2
1	Dichlorvos	7.211	8977117	109	185	79
2	Propamocarb	9.849	3647142	58	71	129
3	Mevinphos	10.828	3540205	127	192	109
4	Chloroneb	13.015	25711940	191	193	206
5	Methomyl	14.837	37353	105	88	57
6	Propachlor	16.350	9464032	120	77	176
7	Propoxur	16.440	9160613	110	152	81
8	Ethoprophos	17.183	4565646	157	97	139
9	Bendiocarb	18.808	6869526	151	126	166
10	Sulfotep	19.350	6463922	322	202	97
11	Alfa-BHC	19.449	12556733	183	181	219
12	hexachlorobenzen	19.949	20260176	284	249	142
13	Dichloran	20.412	5884505	176	206	124
14	Dimethoate	20.694	564535	87	93	125
15	Simazine	21.240	5090199	201	186	173.2
16	Carbofuran	21.517	9548299	164	149	123
17	Lindan	21.953	11122789	219	181	111
18	Fonofos	22.837	13454063	109	137	246
19	Delta-BHC	23.934	10680216	181	219	111
20	Diazinon	24.181	9089119	179	137	152
21	Iprobenfos	25.472	3722626	91	204	122
22	Pirimicarb	26.194	8392242	166	72	238
23	Dichlorfenthion	26.869	14171019	279	223	162
24	Phosphamidon I	27.074	1319730	127	72	264
25	Phosphamidon II	27.105	3159141	127	72	264
26	Chlorpyrifos-Me	27.530	16157392	286	125	288
27	Vinclozolin	27.643	11630240	212	285	187
28	Carbaryl	27.846	9973625	144	115	116
29	Alachlor	28.292	5619050	160	188	146
30	Ronnal	28.738	7653065	285	287	125
31	Metalaxyl	28.894	4025973	206	146	192.2
32	Fenitrothion	30.004	4044914	277	125	109
33	Linuron	30.118	11276099	61	187	124
34	Aldrin	30.417	15385592	66	263	91
35	Thiobencarb	30.794	13756658	100	72	125
36	Malathion	31.310	7412435	127	173	99
37	Fenthion	31.679	6175538	278	125	109
38	Pirimiphos-ethyl	34.279	3930335	318	333	304
39	Capten	34.791	5879858	79	151	114
40	Chlorofenvenphos	35.549	5566491	267	323	269
41	Chlordan-trans	35.990	8009093	373	375	237
42	Alfa-endosulfan	36.919	16381245	239	237	195
43	Nanchlor-trans	37.168	3558446	409	100	237
44	Chlordane-cis	37.311	13019413	375	373	377
45	Disulfoton sulfon	37.670	11474101	213	153	97

cont						
46	Dieldrin	39.172	22384929	79	265	81
47	P,P-DDE	39.688	37789440	246	318	248
48	O,P-DDD	40.321	13372978	235	237	165
49	Endrin	40.920	19517733	263	265	281
50	Beta-endosulfan	41.820	5900664	207	239	195
51	Chlorobenzilate	42.683	5799780	251	139	253
52	P,P-DDD	43.234	11176726	235	237	165
53	Benodanil	43.773	5128741	231	323	203
54	Ethion	44.131	6916401	231	97	153
55	Carbophenothion	45.558	6928433	157	121	125
56	Resmethrin I	48.965	712644	123	171	143
57	Resmethrin II	49.532	3358395	123	171	143
58	Hexabromobenzen	49.772	12452898	551.6	554	549.6
59	Phosmet	50.300	5631889	160	161	77
60	EPN	50.726	4148877	157	169	185
61	Dicofol	50.955	13559731	139	111	251
62	Fenoxycarb	51.040	12507427	255	186	116
63	Tetramethrin II	51.322	6769070	164	123	81
64	Tetradefon	52.029	11921778	159	111	229
65	Mirex	52.460	17148422	272	274	270
66	Furathiocarb	52.620	6870964	163	57	164
67	Amitraz	53.373	6127115	132	121	147
68	Lamda-cyhalothrin	53.770	6887831	181	197	208
69	Azenophos-ethyl	53.882	5662783	132	160	77
70	allethrin I	54.515	2356063	123	181	81
71	allethrin II	54.523	667587	123	181	81
72	allethrin III	54.590	3425395	123	181	81
73	Permethrin I	54.891	2165141	183	163	165
74	Permethrin II	55.111	5182895	183	163	165
75	Comaphos	55.165	7304665	263	226	109
76	Cyfluthrin III	55.855	1480053	163	165	226
77	Cyfluthrin I	55.997	1980594	163	165	226
78	Cyfluthrin IV	56.103	987227	163	165	226
79	Cyfluthrin II	56.162	1878458	163	165	226
80	Cypermethrin II	56.284	1924237	163	165	181
81	Cypermethrin IV	56.424	1156906	163	165	181
82	Cypermethrin I	56.522	1106793	163	165	181
83	Cypermethrin III	56.575	815582	163	165	181
84	Fenvalerate I	57.454	4865105	125	167	281
85	Fenvalerate II	57.716	3328410	125	167	281
86	Deltamethrin	58.440	2418968	253	181	

Table (2): Groups of ions for SIM acquisition.

Group	Time/min	Ions, amu	Dwell time
1	7.212	58, 105, 109, 110, 120, 127, 158, 191	100
2	18.00	87, 91, 109, 151, 164, 176, 179, 181, 183, 201, 219, 283, 322	100
3	26.00	61, 66, 100, 127, 144, 160, 264, 277, 279, 285, 286	100
4	31.50	79, 191, 213, 235, 239, 246, 263, 265, 267, 278, 333, 373, 375, 409	100
5	41.00	123, 140, 157, 160, 207, 231, 235, 251, 323, 552	100
6	50.50	132, 139, 157, 159, 160, 163, 164, 181, 255, 272	100
7	54.00	123, 125, 163, 165, 183, 253, 362	100

Honey samples and analytical procedure

Honey samples were collected from different regions in Kingdom of Saudi Arabia (Riyadh, Qassim, Northern, Southern, Eastern, and Western Regions) during 2011, and from honey stores, which were imported from some countries (Egypt, Yemen, Kashmir, Libya and Pakistan). Ninety one of ripe honey samples were used in this study (65 samples were local and 26 were imported samples). They were represented eight sources of honey flower plants {26 of cedar honey; *Ziziphus pinachristi* (21 local and 5 imported), 29 of acacia honey; *Acacia spp* (26 local and 3 imported), 18 of alfalfa honey; *Medicago sativ*a (12 local and 6 imported), (6 of imported clover honey; *Trifolium alexandrium,* 3 of local *Astragalus spinousus*, 3 of local *Lavandula sp,* 3 of imported nigella honey; *Nigella sativa,* and 3 of imported sugar cane honey; *Saccharums sp*}. They were collected in jars and transferred immediately to pesticides laboratory to detect the pesticide residues. All samples were stored in the dark at 25 ^{o}C. Five grams of honey were ground with 40g anhydrous sodium sulphate and extracted with three portions of 15 ml hexane acetone mix (2:1 v/v) in a column for 2h, and. the final dilution was concentrated with a rotary evaporator. The extract was solubilized in 80µl isooctane and transferred to an injection vial according to the method of Ozlem, (2006**)**.

Recovery experiment

For recovery studies, the honey samples were filtrated and heated at 35^{o}C for 30 min and spiked with 0.4 ml of a working solution containing between (0.1-0.5) µg/ml for each pesticides (depends on the sensitivity of each compound). A small volume of acetone was added to 5 g of samples no. 1, 9, 29 and 52. The mixture was vigorously shaken to achieve a good homogenization and stored at 4 ^{o}C in darkness prior to analysis, for three replications. The limit of detection and recovery data for tested pesticides at different groups were tabulated in Table (3).

Table (3): Recovery data and limits of detection (LOD mg/kg) for pesticides in honey samples.

n	Pesticides	Spiking level ppm.	LOD Ppm	Recovery % for honey samples			
				*(1)	**(9)	***(29)	****(52)
1	Dichlorvos	0.10	0.02	80	96	95	98
2	Propamocarb	0.10	0.04	84	66	78	98
3	Mevinphos	0.50	0.09	86	50	93	66
4	Chloroneb	0.10	0.01	87	94	79	77
5	Methomyl	0.10	0.10	39	35	55	70
7	Propachlor	0.25	0.02	66	81	65	96
8	Propoxur	0.10	0.03	54	77	77	91
9	Ethoprophos	0.10	0.05	67	97	72	64
10	Bendiocarb	0.10	0.01	91	83	63	100
11	Sulfotep	0.10	0.01	84	105	50	100
12	Alfa-BHC	0.10	0.02	100	100	78	95
13	Hexachlorobenzen	0.25	0.05	87	86	66	75
14	Dichloran	0.50	0.03	81	73	32	65
15	Dimethoate	0.10	0.02	65	82	46	43
16	Simazine	0.10	0.01	102	110	63	98
17	Carbofuran	0.10	0.01	100	100	50	72
18	Lindan	0.25	0.01	100	88	58	100
19	Fonofos	0.10	0.06	100	95	56	79
20	Delta-BHC	0.025	0.05	81	74	91	47
21	Diazinon	0.10	0.03	73	91	66	36
22	Iprobenfos	0.10	0.04	104	66	47	93
23	Pirimicarb	0.10	0.05	51	100	71	57
24	Dichlorfenthion	0.10	0.01	48	82	39	63
25	Phosphamidon I	0.10	0.03	95	89	80	100
26	Phosphamidon II	0.10	0.05	63	99	59	66
27	Chlorpyrifos-Me	0.10	0.03	87	97	86	71
28	Vinclozolin	0.10	0.02	106	90	36	88
29	Carbaryl	0.10	0.03	98	98	59	100
30	Alachlor	0.50	0.04	72	92	71	63
31	Ronnal	0.10	0.02	91	81	60	97
32	Metalaxyl	0.25	0.05	109	97	63	102
33	Fenitrothion	0.10	0.07	84	74	45	58
34	Linuron	0.10	0.04	89	89	41	72
35	Aldrin	0.10	0.02	60	90	57	103
36	Thiobencarb	0.10	0.08	59	88	76	49
37	Malathion	0.50	0.03	77	62	81	85
38	Fenthion	0.10	0.03	91	100	58	100
39	Pirimiphos-ethyl	0.10	0.05	105	100	49	93
40	Capten	0.10	0.06	82	69	76	98
41	Chlordan-trans	0.10	0.05	87	98	64	100
42	Alfa-endosulfan	0.50	0.03	48	78	98	107
43	Nanchlor-trans	0.10	0.02	91	100	49	100
44	Chlordane-cis	0.10	0.01	79	84	77	98
45	Disulfoton sulfon	0.25	0.01	84	100	53	97

cont							
46	Dieldrin	0.25	0.02	70	97	96	100
47	P,P-DDE	0.10	0.03	101	99	91	90
48	O,P-DDD	0.10	0.04	55	88	64	67
49	Endrin	0.10	0.01	103	89	44	95
50	Beta-endosulfan	0.10	0.03	81	71	97	63
51	Chlorobenzilate	0.10	0.01	74	80	49	84
52	P,P-DDD	0.10	0.04	84	53	91	49
53	Benodanil	0.50	0.03	100	100	94	73
54	Ethion	0.25	0.03	91	97	47	89
55	Carbophenothion	0.10	0.04	63	100	71	37
56	Resmethrin I	0.10	0.03	49	64	65	71
57	Resmethrin II	0.10	0.04	61	66	49	43
58	Hexabromobenzen	0.10	0.02	106	86	52	59
59	Phosmet	0.10	0.07	88	104	118	71
60	EPN	0.10	0.02	91	70	49	100
61	Dicofol	0.10	0.05	82	100	97	96
62	Fenoxycarb	0.10	0.01	38	82	88	93
63	Tetramethrin II	0.25	0.04	40	75	73	68
64	Tetradefon	0.50	0.03	51	66	77	29
65	Mirex	0.25	0.05	83	67	50	89
66	Furathiocarb	0.10	0.03	61	86	46	55
67	Amitraz	0.10	0.04	75	91	62	69
68	Lamda-cyhalothrin	0.10	0.04	77	74	73	83
69	Azenophos-ethyl	0.10	0.04	85	100	21	77
70	allethrin I	0.10	0.02	60	102	60	67
71	allethrin II	0.10	0.02	77	100	49	102
72	allethrin III	0.10	0.02	89	87	65	91
73	Permethrin I	0.10	0.05	100	99	89	100
74	Permethrin II	0.10	0.04	104	98	99	93
75	Comaphos	0.10	0.04	99	92	101	99
76	Cyfluthrin III	0.10	0.04	93	96	77	89
77	Cyfluthrin I	0.10	0.03	81	99	94	100
78	Cyfluthrin IV	0.10	0.03	93	100	100	102
79	Cyfluthrin II	0.10	0.03	107	93	97	93
80	Cypermethrin II	0.10	0.03	100	100	87	100
81	Cypermethrin IV	0.10	0.02	97	100	84	100
82	Cypermethrin I	0.10	0.02	93	94	105	97
83	Cypermethrin III	0.10	0.05	79	95	59	99
84	Fenvalerate I	0.10	0.03	73	91	66	36
85	Fenvalerate II	0.10	0.04	104	66	47	93
86	Deltamethrin	0.10	0.03	100	100	87	100

Samples no (1, 9, 29 and 52) are random samples as follows:
***Acacia honey collected from Qassim region, Saudi Arabia**
**** Nigella honey imported from Egypt**
*****cedar honey collected from Asir region, Saudi Arabia**
****** Acacia honey collected from Al--Taef province, Saudi Arabia**

RESULTS AND DISCUSSION

A multiresidue procedure was carried out to monitor the pesticide residues in honey during 2011. The honey samples collected from different regions of Saudi Arabia i.e. (Al-Riyadh, Qassim, Eastern Region, Southern Region and Western Region) and other counters (Egypt, Yemen, Libya, Kashmir and Pakistan) were examined. Data in Table (4) shows the amounts of the detected pesticide residues in honey samples collected. The insecticide residues were the majority of the detected chemical compounds. It was found that such insecticides could be classified chemically to its groups, i.e. Organochlorines and Organophosphorues. The most frequent

compounds found were amitraze, coumaphos, endosulfan, α-BHC, malathion, O,P-DDD, P,P-DDD, β-BHC, heptachlor and aldrin. The frequencies for these pesticides were 4, 3, 3, 2, 1, 1, 1, 1, 1 and 1, respectively. The results also indicated that all the honey samples collected from the valleys in Kingdom of Saudi Arabia, like *Ziziphus pinachristi* and *Acacia spp* honey had no pesticides residues detected. Only the residues were detected in honey samples collected from apiaries close to farms may be because those farms, and this may be due to expose of their plants to pesticides which used for controlling the pests.

The results indicated that pesticide residues above maximum concentrations of pesticide residues (MRLs) were detected in 10 honey samples and represented 11% of 91 samples. The samples that had pesticide residues above MRL were (three alfalfa, *Medicago sativ*a local honey collected from bee hives in Riyadh region; one alfalfa, *Medicago sativ*a local honey collected from bee hives Qassim region; three clover, *Trifolium lexandrinum* honey imported from Egypt; two alfalfa, *Medicago sativ*a imported from Pakistan and one alfalfa, *Medicago sativ*a imported from Yemen) (Table 4).

Table (4): Pesticide residues detected and their concentrations in some honey samples.

Source of honey sample	Region	No. of samples contained pesticides residues	Pesticide residues detected in the samples	Concentration of residues (ppm)
Alfalfa honey *Medicago sativ*a	Riyadh	3	Coumaphos	0.001
			Malathion	0.021
			Endosulfan	0.009
			O,P-DDD	0.0031
			P,P-DDD	0.0012
Alfalfa honey *Medicago sativ*a	Qassim	1	Heptachlor	0.002
			Aldrin	0.0009
Clover honey *Trifolium lexandrinum*	Egypt	3	Coumaphos	0.001
			Amitraz	0.021
			α-BHC	0.001
			β-BHC	0.0009
			Amitraz	0.031
Alfalfa honey *Medicago sativ*a	Pakistan	2	α-BHC	0.0011
			Amitraz	0.002
			Endosulfan	0.0033
			Endosulfan	0.0041
Alfalfa honey *Medicago sativ*a	Yemen	1	Coumaphos	0.021
			Amitraz	0.043

*** Maximum concentrations of pesticide residues (MRL) is nil for aforementioned compounds.**

Overall, the pesticides residues found in this study were approximate similar to other studies and with other many methods reported for determination of pesticides in honey, which used against the varroa mite diseases (acaricides and organophosphorous pesticides) or for insect control on numerous field crops (Formica, 1984; Fernandez *et al*.,, 1991; Jimenez *et al*., 1998; Tsipi *et al*., 1999; Albero, *et al*., 2001;).

Bees are always in touch with the environment. Their environment is however often exposed to the pollution with various emissions of harmful industrial substances. Air pollution comes mostly from town-planning activities and traffic. It can affect the flower nectar or the honeydew, when pesticides or other particles of harmful substances enter the composition of these natural sources. Once pesticides were used for controlling crop pests, major risks for honeybees as well as pollution for bee products will be appeared. On the other hand, the Organochlorines pesticides were the most frequently detected in countries used pesticides. Although the use of DDT, HCH, and HCB in Middle East for decades, the results obtained could be expected, because these pesticides and their metabolites have been extensively used and their residues are still present in the environment. Organochlorines are lipophilic substances and consequently are soluble and stable in beeswax. Therefore, an amount of these substances gradually migrates from wax into the stored honey. It is difficult to compare our result with those of other monitoring programs from other countries, because of the range of used pesticides is different. Al-Rifai and Akkel (1997) conducted a monitoring study to determine 50 pesticide residues in 26 honeys from Jordan during 1994 to 1995. They found 86% of the honey samples analyzed were contaminated with organochlorine pesticides. The α-HCH, β-HCH and lindane were the most frequently found. The study performed in 27 honey samples from India during 1993 to 1995 by Anju *et al*., (1997) showed that all honey samples were contaminated by organophosphorus, mainly DDVP, chlorpyriphos, monocrotophos, dimethoate, and fenitrothion. Carbofuran and carbaryl contaminated 55% of the honey samples. All honey samples studied were also contaminated with organochlorines, but the amount of residues found was much lower than that of organophosphorus and carbamates. In Romania, Antonescu and Mateescu (2001) analyzed 265 honey samples and found that the positive samples for α-HCH, β-HCH, lindane and DDT- total were 45%, 39%, 50%, and 25%, respectively. Blasco *et al*. (2004) reported that 14 Valencian honey samples were contaminated, containing residues of HCB or/and HCH isomers. The frequency of detection was 56% for Spanish samples. In Spanish samples, concentrations range from zero to 0.03mg/kg for HCB, and zero to 2.24 mg/kg for HCH-total. In Portugal, 23 samples were contaminated, what means 95.8%. The samples from Portugal showed higher levels. Levels of HCB ranged from zero to 0.39 mg/kg. HCH-total ranged from zero to 4.86 mg/kg and DDT-total from zero to 0.658 mg/kg. Bogdanov *et al*., (2003) searched organo-chlorine, organo-phosphorus and fungicides in 27 honey samples and reported that the results of their study showed that there is no significant contamination in Switzerland. Mukherjee (2009) conducted a study to determine pesticides residues in Indian's honey and found that none of the honey samples analyzed contained the studied compounds at concentrations above the corresponding detection limits.

CONCLUSION

Honey, being a natural product manufactured by honey bees is considered to be free from any extraneous material. The over-reliance on pesticides caused several environmental problems including pesticide residues in food. This constitutes a potential risk for human health, because of their sub acute and chronic toxicity. Therefore, it is imperative to monitor the presence of pesticide residues in honey, to know the extent of pesticide residue present in honey. Obtained data are to be used as a reference point for future monitoring and taking preventive measures to minimize human health risks.

REFERENCES

Albero, B., Sanchez-Brunete, C. and Tadeo, J. L. (2001). Multiresidue determination of pesticides in honey by matrix solid-phase dispersion and gas chromatography with electron-capture detection. Journal of AOAC International, 84(4): 1165–1171.

Al-Rifai, J., and Akkel, N. (1997). Determination of pesticide residues in imported and locally produced honey in Jordan. Journal of Apicultural Research, 36: 155–161.

Anju, R., Beena, K., Gahlawat, S. K., Sihag, R. C. and Kathpal, T. S. (1997). Multiresidue analysis of market honey samples for pesticidal contamination. Pesticide Research Journal, 9: 226–230.

Antonescu, C. and Mateescu, C. (2001). Environmental pollution and its effects on honey quality. Roumanian Biotechnological Letters, 6(5): 371–379.

Beatriz- Albero ; Brunete, C. S. and Tadeo, J. L. (2004). Analysis of Pesticides in Honey by Solid-Phase Extraction and Gas Chromatography−Mass Spectrometry. J. Agric. Food Chem., 52 (19): 5828–5835.

Blasco C., Ferna´ndez M., Pena, A., Lino, C., Silveira, M.I., Font, G. and Pico, Y. (2003). Assessment of pesticide residues in honey samples from Portugal and Spain. J. Agric Food Chem 51:8132–8138.

Blasco, C., Lino, C. M., Pico, Y., Pena, A., Font, G. and Silveira, M. I. N. (2004). Determination of organochlorine pesticide residues in honey from the central zone of Portugal and the Valencian community of Spain. Journal of Chromatography A, 1049: 155–160.

Bogdanov S. (2006). Contaminants of bee products. Apidologie, 37: 1-18.

Bogdanov, S., Ryll, G. and Roth, H. (2003). Pesticide residues in honey and beeswax produced in Switzerland. Apidologie, 34: 484–485.

Codex Alimentarius (1998). Draft revised for honey at step 6 of the Codex Procedure. CX 5/10.2, CL 1998/12-S.

Cruz S., S., Lino, C. M., and silveira, M. I. (2003). Determination of organochlorine pesticide residues in honey from the central zone of Portugal and the Valencian community of Spain Sci. Total Environ. 3017.

Deka S.C., Barman, N., Baruah, A. (2004). Monitoring of pesticide residues in honey samples of Jorhat district, Assam (India). Pestic. Res. J. 16: 83–84.

European Commission Regulation (1990). (EC) No. 2377/90 of 26 June 1990 laying down a Community Procedure for the stablemen of maximum residue limits of veterinary medicinal products in foodstuff of animal origin (as amended by regulations).

European Commission Regulation (1996). ECC No. 2034/96 (OJ L27225.10.1996, p. 2), No2686/98 (OJ L33712.12.1998, p. 20) No. 1931/99 (OJ L240 10.09.1999, p. 3).

European Commission Regulation (1999). No. 239/99 (OJ L 290 12.11.1999, p. 5).

Fernandez M. A., Pico, Y. and Manes, J. (2002). Comparison of solid-phase microextraction and stir bar sorptive extraction for determining six organophosphorus insecticides in honey by liquid chromatography–mass spectrometry J. Food Protect. 65 (11): 1507.

Fernandez M. A., Sancho, M. T., Munitegui, S., Huidobro, J. F. and Smal-Lozano, J. (1995). Nonacaricide Pesticide Residues in Honey: Analytical Methods and Levels Found. J. Food Protect. 58 (11): 1227.

Fernandez Muino, M. A. and Simal Lozano, J. (1991). Simplified method for the determination of organochlorine pesticides in honey. Analyst, 116(3): 269–271.

Food and Drug Administration of the United States (2003). http://www.cfsan.fda.gov.

Formica, G. (1984). Gas chromatographic determination of residues of bromopropylate and two of its degradation products in honey. Journal of the Association of Official Agricultural Chemists, 67(5): 896–901.

Jimenez, J. J., Bernal, J. L., del Nozal, M. J., Martin, M. T., and Mayorga, A. L. (1998). Solid-phase microextraction applied to the analysis of pesticide residues in honey using gas chromatography with electron-capture detection. Journal of Chromatography A, 829: 269–277.

Mukherjee, I. (2009). Determination of Pesticide Residues in honey Samples. Bull Environ. Contam. Toxicol. 83:818–821.

Ozlem erdogrul (2006). Levels of selected pesticides in honey samples from Kahramanmaras, Turkey. Food control: 1-6.

Pang, G, F., Fan, C, L., Liu, Y. M., Cao, Y. Z., Zhang, J. J., Fu, B. L., Li, X. M., Li, Z. Y. and Wu, Y. P. (2006). Multi-residue method for the determination of 450 pesticide residues in honey, fruit juice and wine by double-cartridge solid-phase extraction/gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. Food Additives and Contaminants, 23 (8): 777-810.

Rathi, A., Kumari, B, Gahlawat, S.K., Sihag, R.C. and Kathpal, T.S. (1997). Multiresidue analysis of market honey samples for pesticide contamination. Pestic. Res. J 9:226–230.

Rissatoa, S.R., Galhiane, M.S., de Almeida, M.V., Gerenutti, M. and Apon, B.M. (2007). Multiresidue determination of pesticides in honey samples by gas chromatography–mass spectrometry and application in environmental contamination. Food Chem. 101:1719–1726.

Tsipi, D., Triantafyllou, M. and Hiskia, A. (1999). Determination of organochlorine pesticide residues in honey, applying solid phase extraction with RP-C18 material. Analyst, 124(4): 473-475.

التحليل المتعدد لمتبقيات المبيدات الكيماوية في عسل النحل المستور والمنتج محلياً في المملكة العربية السعودية

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تم جمع عينات عسل النحل من مناطق مختلفة من المملكة العربية السعودية خلال مواسم جني العسل في عامي 2010-2011، لرصد متبقيات المبيدات في بعض عينات العسل المحلي والمستوردة .تم تحديد متبقيات المبيدات بواسطة جهاز الكروماتوجرافي الغازي –مطياف الكتلة حيث تم تطوير طريقة للكشف عن 86 مبيد و التى اظهرات نسبة عالية من معدل الاسترجاع من المبيدات شائعة الاستخدام لحماية المحاصيل المختلفة الطازجة للخضار والفاكهة و التى تختلف في خواصها الكيميائية والفيزيقية (مجموعة المبيدات الفوسفورية – الكلورونية – البيرثرويد – الكربامتية). اظهرت النتائج ان نسبة متبقيات المبيدات التى تجاوزت الحدود المسموح بها وجدت في 10 عينات من العسل والتي تمثل 11% من إجمالي 91 عينة. في حين كانت نسبة العينات الخالية من متبقيات المبيدات تقدر بحوالي 89%. كما اظهرت النتائج ان أكثر متبقيات المبيدات التى وجدت في عينات العسل هي الامتراز وكومافوس واندوسلفان، والتى تمثل مجموعة من المبيدات الاكاروسية التى تستخدم بشكل كبير لمكافحة اكاروس الفاروا في طوائف نحل العسل. وتوصي بأهمية استمرار برامج التقصي عن بقايا المبيدات في اسواق العسل.

قام بتحكيم البحث

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