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ESTABLISHMENT OF ANALYTICAL METHOD AND MONITORING FOR DETERMINATION OF PESTICIDE RESIDUES IN COLLECTED HONEY SAMPLES

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ABSTRACT

Three different analytical methods were used in this investigation to choose the suitable method with high recovery to validate an analytical method for pesticide residues in honey samples.

The systems of GC/NPD and GC/ECD were used to measure recoveries from spiked samples. The honeybee samples free from residues were spiked at one level and the recoveries of pesticides were studied. Spiked level was settled according to the detectors sensitivity to test pesticides in the range of 0.05 – 1.0 µg/g. The three tested analytical methods gave relatively high recoveries for the tested pesticides (84.1-99.5%) with standard divisions varying from ± 8 to 10.7% for different pesticides.

The percent mean recoveries of tested pesticides in the 1st, 2nd and 3rd analytical method were 92.3, 84.1 and 99.5%, with standard deviations ±10.7, 9.8 and 8.0%, respectively. In general, the recoveries obtained with the third analytical method were always higher than those obtained with the first and second analytical methods, with low standard deviations.

All the collected honey samples during two years were free from any pesticides contamination except one sample was obtained at year 2005 from Ehnasia district of Bani Suwayf Governorate. This sample was contaminated with the insecticide chlorpyrifos -ethyl at concentration 0.02 µg/g (below the quantification limit 0.05 µg/g). The presence of residue of such compound in the samples analyzed may be attributed to intensive use of chlorpyrifos -ethyl in cotton fields in controlling the cotton leaf worms and cotton boll worms.

Keywords*:* Food analysis, pesticide residues, gas chromatography, dispersive solid phase extraction, QuEChERS, honey.

INTRODUCTION

Bees are estimated to forage on plants growing in a relatively large area of more than 7 Km^2Celli (1984). If it is assumed that any hive includes at least 1000 worker-bees and that each of them forage on one thousand flowers per day, the honey produced daily can be considered the out-come of at least one million interactions Buldini *et al.(* 2001). In this way, the forage area is effectively sampled for pesticides residue in honey reflects their levels in the forage area. From early times honey was considered a delicious food, but it is also the result of a bio-accumulative process useful for collecting information about the environment within the bees forage area.

The extensive use of pesticides to improve agricultural productivity has resulted in the wide distribution of these compounds in the environment. It is important to develop analytical methods for detection and quantitative determination of pesticide residues in honey comprising minimum extraction

and clean-up steps. Chromatographic analysis follows usually after tedious sample preparation where compounds are extracted from the complex materials. There are various extraction and clean – up procedures for isolation of pesticides residue from food and environmental samples. Almost all traditional methods (shake-flask, Soxhlet etc.) are time and solvent consuming, while the extraction yield is often insufficient. A number of methods, such as ultrasonic solvent extraction, solid phase extraction (SPE) and supercritical-fluid extraction (SFE) are proposed to resolve the solvent consumption problem Tekel & kovscicova(1993)and Pacakova *et al.*(1996). In honey analysis there are liquid-liquid extraction (Martel and Zeggane, 2002) as well as solid – phase micro extraction (Jimenez *et al.,* 1998) and florisil column extractions used for isolation of pesticides. This work aimed to develop a rapid and simple multiresidue method to determine and confirm eighteen pesticides of different chemical classes; the choice analytical method was used in determination of the percent recovery of forty six pesticides from spiked honey and detection the contamination in collected honey samples from apiaries localized in three different Governorates in Egypt (El Sharquia, El Fayum and Bani Suwayf) during years 2005 and 2006.

MATERIALS AND METHODS

Chemicals

The tested eighteen pesticides belong to three categories; twelve insecticides, three acaricides and three fungicides. They were selected on the basis of their commercially use in the control of different insects, fungi and mites, and also due to their long persistence in environment such as organochlorines compounds. Insecticides are the main group of pesticides that have caused incidents of poisoning of honeybees. The chosen insecticides represent different chemical families; three organochlorines, seven organophosphorus, one carbamets and one pyrethroides.

All reagents should be of analytical (HPLC) grade; acetonitrile, acetone and hexane from (Lab-scan) (HPLC, assay >99%).

- Tetradecane (HPLC, assay >99%), acetic acid (glacial) and anhydrous magnesium sulphate (Merck).
- Primary secondary amine bulk sorbent (PSA) and sodium acetate (Riedel-deHäen).
- De-ionized water was produced by mille Q unit (Mille Pore).
- Acetonitrile acidified with acetic acid (1%): prepared by adding 1.0 ml glacial acetic acid in 100 ml acetonitrile

Standard preparation

A stock solution: Pesticide reference standards from Dr. Ehrensdorfer (Augsburg, Germany), were >95% Purity. 100 µg/ml reference standard solutions of all the analyzed pesticides were prepared in hexane:acetone (9:1) in 100 ml volumetric flask. Stock solution was kept in refrigerator at 4 ± 2^{o}C.

Intermediate solution: individual standards of 10 µg/ml of pesticides were prepared by diluting 5.0 ml of stock solution in 50 ml hexane. Intermediate solution was kept in refrigerator at 4 ± 2^{o}C.

Calibration solutions: Calibration mixtures were divided into nitrogen-phosphorus detector (NPD) and electron capture detector and prepared by dissolving appropriate amounts of intermediate solutions in hexane: acetone (9:1).

Internal standard solution. Mixture of aldrin (0.1 µg/ml) and ditalimfos (0.3 µg/ml) were prepared in hexane:acetone (9:1) solution for GC-NPD and GC-ECD.

System suitability test (SST) mixture: The operation conditions of the chromatographic system were monitored with properly selected system suitability test (SST) mixtures, which provide information with one injection on the characteristic performance parameters of the whole system from the injector to the detectors. The system suitability mixture is injected every day and the GC performance is monitored by evaluating the tailing, resolution, number of theoretical plates, asymmetry, sensitivity, selectivity and detection limit.

Matrices and real samples

Honey samples were collected from three different Governorates; El Sharquia, El Fayum and Bani Suwayf during years 2005 and 2006 (Table 1). El Sharquia Governorate is representative the north Egypt (Delta region) and El Fayum & Bani Suwayf Governorates are representative the mid Egypt.

Table (1): Locations, numbers and varieties of collected honey samples during years 2005 and 2006

Governorate	District	2005		2006	
		No. of sample	Crop	No. of sample	Crop
El Sharquia	El Zagazik	2	Cotton	2	Cotton
		1	Clover	1	Clover
	Diarb Negm	1	Cotton	1	Cotton
		1	Clover	1	Clover
El Fayum	El Fayum	2	Cotton	2	Cotton
		1	Clover	1	Clover
	Esta	2	Cotton	2	Cotton
		2	Clover	2	Clover
	Sanoris	2	Cotton	2	Cotton
		1	Clover	1	Clover
Bani Suwayf	Bani Suwayf	2	Cotton	2	Cotton
		1	Clover	1	Clover
	Ehnasia	2	Cotton	2	Cotton
		1	Clover	1	Clover
	Naser	2	Cotton	2	Cotton
		2	Clover	2	Clover

All honey samples were collected from apiaries near of cotton or clover fields in the three Governorates. Twenty five samples of honey were collected from the Districts; El Zagazik (3) and Diarb Negm (2) in El Sharquia Governorate, El Fayum (3), Etsa (4) and Sanoris (3) in El Fayum Governorate and Bani Suwayf (3), Ehnasia (3) and Naser (4) in Bani Suwayf Governorate during year 2005. On the other hand, this honey samples were divided to 15 samples was collected from apiaries near of cotton fields and 10

samples from apiaries near of clover fields. The collected honey samples in year 2006 were identical with those obtained in year 2005. The third analytical method was used to determine the pesticide residues in collected honey samples.

Methods of analysis

Method (1)

The analytical method of Porter and Burke (1969) for extraction and clean up of pesticide residues from honey was used in the present study. Honey sample (100 g) was spiked with tested pesticides. The heated mixture of 200 ml acetonitrile and 50 ml de-ionized water at 75ºC was used for extraction the tested pesticides and florisil column chromatography was used for purification of the extract. Forty five milliliter of the first elute (n-hexane: benzene: ethyl acetate 180:19:1) were used at flow rate about 2.5 ml/min and then, twenty five milliliter of the second elute (ethyl acetate: n-hexane 3:7) was added to florisil column before exposure the sodium sulphate layer to air. The total elutes were collected and evaporated till dryness, then 5 ml of injection standard was added and the pesticide residues were determined using GLC.

Method (2)

Honey sample (20 g) was dissolved in 20 ml de-ionized water by using homogenizer for one minute and the solution was extracted with 100 ml ethyl acetate in the presence of 30 gm sodium sulphate using homogenizer for two min. the extract was filtrated through filter paper No. 1. The filtrate was evaporated using rotary evaporator to dryness and re-dissolved in 8 ml mixture of n-hexane : ethyl acetate (1:1). One ml of this extract equivalent to ca 2.5 g honey was purified on a gel permeation column with ethyl acetate – cyclohexane (1:1) as mobile phase at flow rate 1.5 ml/min. The pesticide fraction (22 ml) was evaporated to dryness and dissolved in 2 ml of injection standard. The final solution was equivalent ca 1.25 g honey/ml. This method was described by Jansson,(2000)

Method (3) QuEChERS Method

The procedure Lehotay *et al.*,(2005)was used for extraction and purification of pesticide residues from honey samples as described below. Honey sample (5 g) was weighted into a 50 ml PFTE tube and dissolved in 10 ml deionized water by shaking for one minute. Acetonitrile acidified with acetic acid (10 ml), 1.0 g sodium acetate and 4.0 g anhydrous magnesium sulphate were added and shake vigorously for one minute. The samples were centrifuged at 4000 rcf for 2 min. Six milliliter of the upper clear solution (extracts) was transferred into 15 ml polyethylene tube contain 0.4 g primary secondary amine (PSA) sorbent and 0.6 g anhydrous magnesium sulphate. The tubes were caped, then the extract with the sorbent/dessicant mixed vigorously for one minute and centrifuged at 4000 rcf for 2 min. Four milliliter of the clear solution was transferred into 15 ml glass tube and 50 µl tetradecan was added as keeper and evaporated in turbovab at 40 ^{o}C to dryness. The residues were dissolved in 2 ml of injection standard and 1 µl of the sample was injected into GC-NPD and GC-ECD.

GLC conditions

Gas Chromatography equipped with two NP detectors were used for detection of organophosphorus and organonitrogen pesticides (Agilent 6890 series) and other GC equipped with two EC detectors were used for detection of organochlorine and pyrethroid pesticides. For results confirmation, samples were injected into two capillary columns with different polarities (HP-PAS-5: 0.32 mm x 0.52 µm x 25 m and DB-1701P: 0.32 mm x 0.25 µm x 25 m).

Gas liquid chromatography was used for determination of pesticide residues in honey samples under the following conditions: N2 constant flow, 1.3 mL/min; inlet temperature, 225°C; injection volume, 1 µl (splitless); initial oven temperature, 90°C, held for 2 min, then a 20°C/min ramp to 150°C followed by a 6°C/min ramp to 270°C (held for 18 min).These conditions were used for the two GLC apparatus.

Calculations

Analyte concentration in sample Cs (mg/kg) is calculated as follows:

$$Cs = Ci \times \frac{Vtot}{Ve} \times \frac{Vf}{W}$$

Ci = Concentration in injection (µg/ml).
V_{tot} = Total volume of extract (ml).
V_e = Volume of aliquot taken for evaporation (ml).
V_f = Final volume (ml).
W = Sample weight (g).

RESULTS AND DISCUSSION

Development of analytical method

In order to analyze a large number of pesticides from different classes and minimize the effect of honey extractive and clean up on the determination of the pesticides studied, as well as to improve good recoveries, three analytical methods have been tested. For the purpose of comparison, 5 g honey sample was employed in the third analytical method instead of 100g in the first and 20 g in the second method. The most important consideration, however, relates to sample size. If a large sample size is need, then larger tubes, centrifuges and rotors and more materials are required, which leads to a domino effect of greater expense. The 3rd method requires little amount of solvents and samples. A remarkable advantage of the third analytical is that the isolation and purification are combined into one step. The time required for sample preparation, extraction and clean up by the traditional methods (1st and 2nd analytical methods) was much longer compared with the 3rd analytical method.

Recovery experiments were performed in order to study the accuracy of each analytical method. The systems of GC/NPD and GC/ECD were used to measure recoveries from spiked samples. The honeybee samples free from residues were spiked at one level and the recoveries of pesticides were studied. Spiked level was settled according to the detectors sensitivity to test pesticides in the range of 0.05 – 1.0 µg/g. The three analytical methods gave

relatively high recoveries for the tested pesticides (84.1-99.5%) with standard divisions varying from ± 8 to 10.7% for different pesticides. The percent recoveries of tested pesticides in the 1st, 2nd and 3rd analytical method were 92.3, 84.1 and 99.5%, while their standard deviations were ±10.7, 9.8 and 8.0%, respectively (Table 2). The lower recovery percentage was obtained with fenitrothion (75%) using the 2nd method, with bupirimate (79%) using the 1st method and parathion methyl (85%) using the 3rd method. In contrary, the highest recovery percentage was obtained with fenvalerate (115%) using the 1st method, bromopropylate (113%) using the 3rd method and gamma-HCH (112%) using the 2nd method. Also, the percent recoveries of tested pesticides belong to organochlorine group in the 1st, 2nd and 3rd analytical method were 86.2, 91.8 and 102.6%, while their standard deviations were ±7.3, ±16.0 and ±5.5%, respectively. In case of organophosphorus insecticides, the percent recoveries in the 1st, 2nd and 3rd analytical method were 94.9, 82.0 and 98.7%, while their standard deviation were ± 9.9, ± 5.3 and ± 7.7%, respectively. In general, the recoveries obtained with the third analytical method were always higher than those obtained with the first and second analytical methods. In the same way, the standard deviations of percent recovery for tested pesticides in 3rd analytical method were always lower than those obtained with the 1st and 2nd analytical methods.

Table (2): Recoveries (%) of the tested pesticides in honey samples using three different analytical methods.

No.	Common name	Added conc. (µg/g)	Recovery (%) in the analytical methods		
			(1)	(2)	(3)
Insecticides					
1	gamma- HCH	0.05	80	112	96
2	delta- HCH	0.10	77	97	104
3	*op*`DDE	0.05	94	78	109
4	Chlorpyrifos-ethyl	0.30	112	---	105
5	Dimethoate	0.30	102	86	---
6	Fenitrothion	0.30	99	75	105
7	Malathion	0.30	86	87	95
8	Parathion-methyl	0.30	92	76	85
9	Pirimiphos-methyl	0.30	88	86	100
10	Profenofos	0.30	85	82	102
11	Pirimicarb	0.30	85	---	89
12	Fenvalerate	0.10	115	79	99
Acaricides					
13	Dicofol	0.10	90	---	106
14	Tetradifon	0.10	90	80	98
15	Bromopropylate	0.10	98	80	113
Fungicides					
16	Bupirimate	1.00	79	---	97
17	Procymidone	0.30	88	79	84
18	Vinclozolin	0.05	102	81	104

Detection of pesticide residues in honey samples:

Before detection the pesticide traces in collected honey samples from the different governorates, the recovery percentage and trueness of forty six pesticides was conducted by 3rd analytical method

Recovery test

Data in Tables (3) shows the mean recovery and precision obtained (relative standard deviation) from spiked samples. The added concentrations of tested pesticides were ranged from 0.02 to 1.0 µg/g. Recoveries were between 84.2% for profenofos and 120.3% for parathion-ethyl and pirimiphos-ethyl. On the other hand the relative standard deviation ranged between ±1.1% for alpha HCH and ±14.6% for phosalone. The percent recovery was excellent for all tested pesticides except for gamma HCH, profenofos, triazophos and atrazine was satisfactory, which reached 86.7, 84.2, 89.3 and 86.8%, respectively.

Trueness

The trueness of a method is an expression of how close the mean of a set of results (produced by the method) is to the true value. The method trueness was confirmed by participation in interlaboratory comparison with Finish Customs Laboratory, Espoo, Finland. Honey sample was spiked at Finnish laboratory and sent to our laboratory as blind sample, the sample was analyzed using the developed method. The z-scores were calculated by Finnish laboratory using the spike level as true value and 25% as target standard deviation. The results of the interlaboratory comparison are shown in Table (4). It was found that the obtained z scores within the approval limit.

Monitoring the pesticide residues in collected honey samples

A few works on the monitoring of pesticide residue levels in honey have been previously published in the literature. The methods are limited to determine acaricides and insecticides widely used in hives Martel and Zeggane(2002),Jimenez *et al.(* 2002)and Blasco *et al.*,(2004) or only to evaluate one pesticide class Blasco *et al.*(2004). A recent multiresidue pesticides method developed for 15 organohalogen pesticides, six polychlorinated biphenyls and seven organophosphours pesticides was implemented for routine determination of residues in honey, showing low levels of pesticides in real honey samples (Herrera *et al.,* 2005). Also, (Rissato *et al.,* 2007) developed a simple and fast multiresidue method to determine 48 pesticides within the majour groups of pesticides (organohalogen, organophosphours, organonitrogen and pyrethroids) in honey samples. The results was indicated that most pesticide residues found in the samples belong to the organohalogen and organophosphours groups and lower levels of residues of some organonitrogen and pyrethroids were also detected.

The present work, developed multiresidue method to determine 46 pesticides belong to the major chemical groups. The applicability of the method to routine analysis was continuously assessed by analyzing fifty honey samples, which were collected from three different Governorates; El Sharquia, El Fayum and Bani Suwayf during years 2005 and 2006. The honey samples were taken from different apiaries near intensive horticulture

area (cotton and clover fields) and analyzed using the accredited method of analysis Barakat *et al.* (2007).

Table (3): Recovery percentage and relative standard deviation of tested pesticides

No	Common name	Expected (µg/g)	Mean recovery (%)	Stand. deviation (%)	Relative Standard deviation (RSD%)
			Insecticides		
1	Alpha- HCH	0.02	90.80	1.00	1.10
2	beta- HCH	0.03	103.0	10.1	9.80
3	gamma- HCH	0.02	86.70	5.40	6.30
4	delta- HCH	0.05	101.3	6.00	5.90
5	Alpha- Endosulfan	0.02	108.3	6.90	6.40
6	beta -Endosulfan	0.02	110.7	7.40	6.70
7	*p,p`*DDD	0.02	108.5	6.10	5.60
8	p,p`DDE	0.02	96.70	6.80	7.00
9	Dieldrin	0.02	114.5	2.90	2.50
10	Endrin	0.05	110.2	4.30	3.90
11	Heptachlor	0.02	91.40	12.9	14.1
12	Heptachlorepoxid	0.02	110.5	5.70	5.20
13	Chlorpyrifos-ethyl	0.05	105.3	6.70	6.30
14	Chlorpyrifos-methyl	0.05	96.70	1.60	1.70
15	Diazinon	0.05	97.20	1.70	1.80
16	Fenitrothion	0.05	113.2	6.50	5.70
17	Fenthion	0.05	97.00	6.20	6.40
18	Malathion	0.05	93.30	1.90	2.00
19	Parathion-ethyl	0.05	120.3	8.10	6.70
20	Parathion-methyl	0.05	91.80	4.00	4.30
21	Phosalone	0.05	91.80	13.4	14.6
22	Pirimiphos-ethyl	0.05	120.3	8.10	6.70
23	Pirimiphos-methyl	0.05	95.00	11.1	11.7
24	Profenofos	0.05	84.20	14.6	17.4
25	Prothiofos	0.05	103.4	5.10	5.00
26	Triazophos	0.05	89.30	3.80	4.30
27	Bendiocarb	0.10	101.8	7.50	7.40
28	Carbosulfan	0.05	107.3	5.60	5.20
29	Pirimicarb	0.05	108.5	5.10	4.70
30	Alpha-Cypermethrin	0.20	103.7	5.20	5.00
31	Cyfluthrin	0.05	101.7	10.3	10.2
32	Fenvalerate	0.05	103.0	4.410	4.30
33	Lambda-Cyhalothrin	1.00	109.8	2.30	2.10
34	Permethrin	0.30	106.5	4.50	4.20
			Acaricides		
35	Dicofol	0.07	106.0	5.80	5.40
36	Tetradifon	0.05	109.8	9.60	8.70
37	Bromopropylate	0.05	97.70	7.50	7.70
			Fungicides		
38	Bupirimate	0.05	105.3	3.40	3.30
39	Chlorothalonil	0.02	109.7	8.60	7.80
40	Pyrazophos	0.05	96.80	7.00	7.20
41	Tolclofos-methyl	0.05	104.3	9.80	9.40
42	Iprodione	0.20	104.8	12.1	11.6
43	Procymidone	0.05	106.8	6.00	5.70
44	Vinclozolin	0.02	114.5	4.20	3.70
			Herbicides		
45	Atrazine	0.05	86.70	7.50	8.70
46	Trifluralin	0.02	105.8	7.10	6.70

Table 4: Interlaboratory comparison between Egyptian and Finnish laboratories.

Pesticide	Egyptian Lab. found concentration (µg/g)	Finnish Lab. spike level (µg/g)	z-score*
Chlorpyrifos	0.150	0.164	-0.3
Profenofos	0.305	0.328	-0.3
Heptachlor-epoxide	0.143	0.164	-0.5
Dieldrin	0.142	0.164	-0.5

***Accepted z-score: -2≤ Z ≤ 2**

Data showed all the collected honey samples during two years were free from any pesticides contamination except one sample was obtained at year 2005 from Ehnasia district of Bani Suwayf Governorate. This sample was contaminated with the insecticide chlorpyrifos ethyl at concentration 0.02 µg/g. The presence of residue of such compound in the samples analyzed may be attributed to intensive use of chlorpyrifos ethyl in cotton fields in controlling the cotton leaf worms and cotton boll worms. Accordingly, the percent contamination of collected honey samples over a 2-year period reached 2% only.

Similar finding was reported by El-Nabarawy *et al.* (2001), who detected chlorpyrifos ethyl at the range of 0.041 and 0. 273 ppm in some collected honey samples from different markets of Gharbia Governorate. They reported that the contamination of some collected honey samples with chlorpyrifos ethyl may be due to that this compound and other organophosphorus insecticides were commonly used in the Gharbia Governorate against different insect pests on apple and grapes. Also,(Blasco *et al.*, 2004), reported that the extensive use of organophosphorus pesticides in agricultural practice is the reason of why residues of these pesticides contaminate bees during polling process and are transferred by them into honey.

The maximum concentration of pesticide residues in honey is not included in the Codex Alimentarius and the absence of maximum residue limits (MRLs) makes it difficult to ascertain whether a product is safe for consumers. Up to now, European Union (EU) legislation has established the MRL in honey for only three acaricides; amitraz, coumaphos and cymiazol, as follows; 0.2, 0.1 and 1 µg g^{-1}, respectively. Furthermore, the United States Environmental Protection Agency (USEPA) has established MRLs for amitraz, coumaphis and fluvalinate as follows; 1.0, 0.1 and 0.05 µg g^{-1}, respectively Otero *et al.*(2006).

Consumers are exposed to pesticides, usually in minute quantities, in several food groups including fruits, juices, honey and vegetables. The monitoring pesticide residues in honey help to assess the potential risk of this product to consumer's health and providing information on the pesticides which have been used in the field crops surrounding the hives. As a result, there were some works about the monitoring of pesticide residues in honey samples in different countries. In a monitoring study conducted to determine 50 pesticide residues in 26 honeys from Jordan from 1994 1995 Al-Rifai and Akkel (1997), 86% of the honeys analyzed were contaminated with

organochlorine pesticides with alpha HCH, beta HCH and gamma HCH being the most frequently found. The study performed in 27 honey samples from India from 1993 to 1995 (Anju *et al.*, 1997) showed that all samples were contaminated by organophosphorus, mainly DDVP, chlorpyriphos ethyl, monocrotopfos, dimethoate and fenitrothion. Carbofuran and carbaryl were 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. Of 265 honey samples analyzed from 1999 to 2000 in Romania Antonescu and Mateescu(2001) and the positive samples for alpha HCH, beta HCH, gamma HCH 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 Portugal, 23 samples were contaminated, what means 95.8%. In Spanish samples, concentrations range from 0.0 to 0.03 mg/kg for HCB and 0.0 to 2.24 mg/kg for HCH-total. The samples from Portugal showed higher levels of organochlorine pesticides. Levels of HCB ranged from 0.0 to 0.39 mg/kg, HCH-total ranged from 0.0 to 4.86 mg/kg and DDT-total from 0.0 to 0.658 mg/kg. Bogdanov *et al.*(2003) searched organochlorine, organophosphorus and fungicides in 27 honey samples and reported that the results of their study showed that there is no significant contamination in Switzerland. In Brazil,(Rissato *et al.*, 2007), reported that the residues of organohalogen (endosulfan sulfate, hexachlorobenzene and tetradifon) and organophosphorous (atrazine, simazine and tebuconazole) pesticides were found at low level of contamination in most honey samples studied. In contrary, malathion was detected in higher concentrations, as compared to others, due to application of this pesticide in the control of dengue mosquitoes.

Erdogrul ,(2006) reported that there is not a significant contamination source for honey production in Kahramanmaras region of Turkeyl. In Spain, Campillo *et al. (*2006) confirmed that none of the honey samples analyzed contained the studied compounds (16 pesticides) at concentrations above the corresponding detection limits. Botitsi *et al.*(2006) analyzed 312 honey samples, which collected from Greece market to monitoring the residues of 1,4 –dichlorobenzene (p-DCB). Of the 312 samples so far, 44% were found with no detectable residues of p-DCB, in 26% of the samples, the concentration of p-DCB was less than 10 µg kg^{-1}, in 24% of the samples the concentration of p-DCB was in the range 10 - 40 µg kg^{-1}, while 6% of the sample contained more than 40 µg $kg^{-1}$of p-DCB. Non-compliant honey samples were withdrawn from the market. All the samples labeled as biologically produced honeys were found free from p-DCB. Eleven Portuguese commercial honey samples were analyzed by (Otero *et al.*, 2006) to determine whether the concentration of acaricides in honey exceed their maximum residue limits (MRLs). Acaricide residues detected were lower than those established by legislation.

It is difficult to compare the present results with those of the other monitoring programs from other countries, because there are only a few of them published, the range of pesticides considered is different and the honey

contamination in Egypt is extremely lower than those in the other countries. These results are the first data regarding the honey contamination in three different regions. But, further and more extensive studies are necessary that included much more areas.

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تطوير طرق تحليل وتقصي مستوى متبقيات المبيدات في عينات عسل النحل

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ثلاث طرق لتحليل متبقيات المبيدات فى العسل تم استخدامها فى هذه الدراسة لأختيار الأمثل منها من حيث الكفأه العالية فى الأستخلاص ومعدل الأسترجاع العالى وذلك ليتم أجراء دراسة أثبات الكفائه على الطريقة المختاره وذلك لتقدير متبقيات المبيدات فى العسل . اجريت تجارب معدل الأسترجاع وذلك للوقوف على دقة كل طريقة من من الطرق المختبرة .

تم أستخدام أجهزة الكروماتوجرافي الغازي لتقدير معدل الأسترجاع فى عينات ملوثه بالمبيدات المستخدمة فى الدراسة عن طريق استخدام عينات عسل خالية من المبيدات والقيام بأضافة الكمية المطلوب استرجعها من المبيدات المختلفة لقياس كفائة الطريقة ويتم تحديد التركيزات المراد درساتها من المبيدات طبقا لحساسية الكاشف المستخدم في الجهاز فكانت المبيدات المختبرة تركيزاتها تتراوح ما بين 0.05-1.0 ميكروجرام/جرام . أعطت الطرق التحليلية الثلاثة المختبرة معدل استرجاع عالي نسبيا يتراوح بين (84.1-99.5 %) مع انحراف معياري من ± 8 إلى 10.7 % للمبيدات المختبرة .

وقد وصل متوسط معدل الأسترجاع للمبيدات المختبرة للطرق التحليلية الأولى والثانية والثالثة الى 92.3, 84.1 و99.5 %، بالإنحرافات المعيارية ±10.7, 9.8 و8.0 %، على التوالي وعموما، كان معدل الأسترجاع بالطريقة التحليلية الثالثة أعلى دائما من معدلات الأسترجاع بالطرق التحليلية الأولى والثانية مع أنحراف معياري منخفض.

وقد أوضحت التجارب أيضا خلو جميع عينات العسل التي تم جمعها خلال سنتي الدراسة من المبيدات ماعدا عيّنة واحدة تم جمعها في سنة 2005 من منطقة إهناسيا محافظة بني سويف. هذه العيّنة كانت ملوثة بمبيد (كلوربيريفوس أثيل) بتركيز 0.02 مليجرام/جرام. ويفسر وجود متبقيات هذا المركّب إلى استعمال هذا المبيد في حقول القطن لمكافحة ديدان ورق القطن وديدان اللوز بكثافة عالية.

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