

DETECTION OF SOME PESTICIDES RESIDUES IN RAW MILKEL ASUOTY, M.S.¹; FAYZA, A. EL TEDAWY²; AMANY, A. SALLAM² and FAYZA, A. SDEEK³¹ Researcher of Food Hygiene, Animal Health Research Institute, Damanhour Branch, Egypt.² Senior Researcher of Biochemistry, Animal Health Research Institute, Damanhour Branch, Egypt.³ Researcher of Pesticides, Department of Pesticides Residues and Environment Pollution., Central Agriculture Pesticides Laboratory, Dokki, Egypt.**Received:** 16 February 2017; **Accepted:** 20 March 2017**ABSTRACT**

Milk could be contaminated with pesticides from fodder, soil and ingestion of insecticides due to widespread application of pesticides in agriculture. Therefore, the present study was conducted to determine the concentration of organochlorine (OC), synthetic pyrethroids and organophosphorus (OP) pesticide, using chromatography mass spectroscopy in milk samples collected from El-Beheria governorate, Egypt. The obtained results revealed that OCPs Hexachlorocyclohexane isomers Alph, gama & Delta (HCHs), Heptachlorepoide, Aldrin, Y- chlordane, Dieldrin, DDE, DDD and Methoxychlor and were monitored at percent of 3.3, 13.3, 83.3, 93.3, 3.3, 13.3, 23.3, 60, 70 and 83.3 % respectively, in the examined milk samples while Heptachlor, Endosulfan, Endrin and DDT were not detected in the same samples. Synthetics pyrethroids including (cypermethrin, deltamethrin, Fenvaluate, Lambda_cyhalothrin and Permethrin) were detected at 60, 40, 50, 56.7 and 60 % of examined samples while, Fenopropathrin was not detected in the examined milk samples. Concerning OP pesticides, all examined milk samples were contaminated with Diazinon 30 (100%), while Chlopyrifos was detected in only one sample 1 (3.3%), other OP pesticides were not detected in the examined samples. This study investigated only pesticides residues and illustrated the risks of pesticides residues exposure that threaten consumer health. Thus, monitoring survey of pesticide residues in milk is necessary to safeguard consumer health.

Key words: organochlorine, Pyrethroid, organophosphorus pesticides, raw milk, Chromatography mass spectroscopy.

INTRODUCTION

Milk is an ideal fluid to dissolve environmental organic pollutants such as pesticide residues since most of them have lipophilic properties (Kampire *et al.*, 2011). Thus, milk is used as an indicator for determining the persistence of organic pollutants. The term pesticide covers a widerange of compounds including insecticides, herbicides, rodenticides, and others. Among these, organochlorine (OC) insecticides, used successfully in controlling a number of diseases, such as malaria, and typhus. The introduction of other synthetic insecticides, i.e., organophosphate (OP) insecticides in pyrethroids and herbicides contributed greatly in pest control and agricultural output (Akhtar *et al.*, 2009).

The contamination of milk is considered as one of the main dangerous issues in the last few years. Milk can be contaminated by pesticide residues such as organochlorine (OC), organophosphorous (OP) pesticides as well as pyrethroids pesticides (Fontcuberta *et al.*, 2008). The presence of pesticide residues in milk is considered a public health concern because milk and dairy products are widely consumed by infants, children, and adults. Pesticide residues in milk originate from contaminated feed, grass or corn silage, and direct application of pesticides on dairy cattle. Because humans are the last link in the food chain, they consume the highest levels of these compounds. Food products particularly milk and dairy products are the primary immediate sources of OC and OP pesticide intake in the general population (Johansen *et al.*, 2004).

Organophosphorus (OP) pesticides are esters, amides, or thiol derivatives of phosphoric acid. OP pesticides are easily hydrolyzed and therefore, do not persist in the environment. However, their toxicity (high or moderate) and the possibility of their accumulation especially fat-soluble OPs in animal tissues, milk, and

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eggs pose risks for human health (Fagnani *et al.*, 2011).

Organochlorine (OC) compounds are lipophilic and metabolized very little in living organisms. Hence, environmental exposure to these compounds results in their accumulation and persistence in fat tissue (Falandysz *et al.*, 2004). Moreover, OC pesticides undergo biomagnification through the food chain causing a chronic toxicity after long-term exposure (Borga *et al.*, 2001). The chronic effects of pesticides from contaminated food on human health are not well defined, but there is increasing evidence of carcinogenicity and genotoxicity, as well as disruption of hormonal functions (Ledoux, 2011).

Organophosphorus and pyrethroids pesticide residues are widely used in agriculture and in animal husbandry in Egypt while, the OC pesticides have been banned a long time ago. OP and pyrethroids pesticides are less persistent than OC pesticide residues in environment. In order to ensure the safety of milk for the consumer, it is very important to know the pesticide residual levels and compared with recommended toxicological criteria such as maximum residue limit (MRL) and the acceptable daily intake (ADI). Legislation in the European Union has established MRLs of 40 ng/g and 0.8 ng/g for the target OC pesticides dichloro-diphenyl-trichloroethane and endrin, respectively, in milk. The MRL for chlorpyrifos is 10 ng/g (EU, 2007).

To aid in the protection of consumer health and elimination of unsafe food, as well as to be comply with the requirements of the international food standard. This study was conducted to determine the

levels of several OC, pyrethroids and OP pesticide residues in raw milk samples collected from various regions of El-Beheriagovernorates.

MATERIALS AND METHODS

1. Samples:

A total of 30 fresh raw milk samples (500 ml of each) were collected from different supermarkets at El-Beheria Governorate, Egypt during the period from April 2016 to May 2016. Each sample was received in glass bottles and tested for heat treatment (storch test) according to (Lampert 1975), then samples was stored at -20°C until for detection of pesticides residues.

2. Pesticide residues analysis according to Lehotay *et al.* (2005):

All reagents and chemical used in this study were of analytical grade. Pesticide standards were used in monitoring survey contained the following OCPs: Hexachlorocyclohexane (HCH) (α -, β -, γ - HCH isomers), heptachlor and its metabolite (heptachlor epoxide), Aldrin, endrin, γ -chlordane, dichlorodiphenyltrichloroethane (pp'-DDT) and its metabolites (pp'-DDE and pp'-DDD) and methoxychlor. While, OP pesticide standard included: ethoprophos, phorate, diazinon, dimethoate, pirimiphos-methyl, chlorpyrifos, malathion, prothiophos, fenamiphos, ethion and triazophos. In addition, the synthetic pyrethroids standard was permethrin, cypermethrin, deltamethrin, Fenopropathrin, Fenvaluate and Lambda_cyhalothrin.

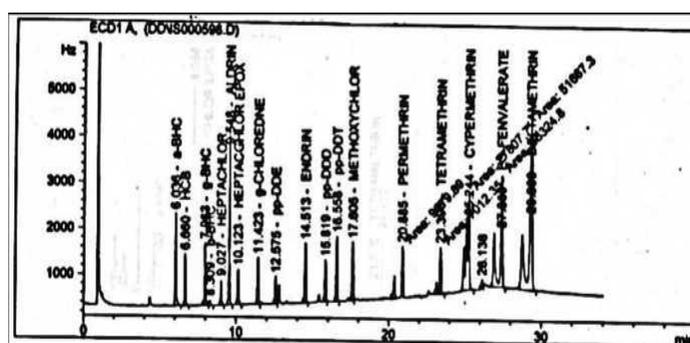


Fig. Organochlorine and pyrethroids mixture reference standard chromatogram

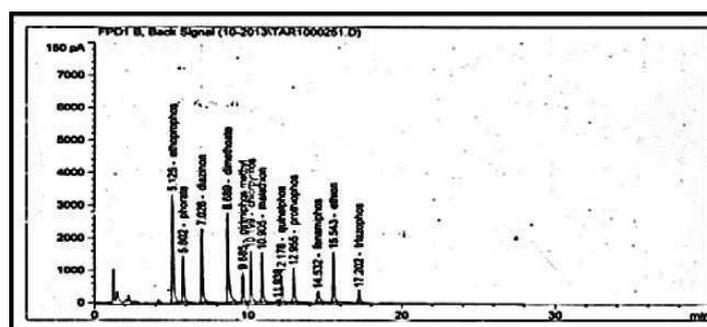


Fig. Organophosphorus reference standard chromatogram

3. Instrument Conditions according to Lehotay *et al.* (2005):

Determination of OC and pyrethroid pesticide residues were performed on Hewlett Packard GC model 6890 N equipped with a Ni63- electron capture detector (GC-ECD). GC-ECD had a capillary column PAS-5 (30 m length x 0.32 mm internal diameter x 0.25 μ m film thickness). Injector and detector temperature program were 300°C for 2 min, raised at the 3°C min⁻¹ and then held at 260°C for 15min. The carrier gas was N₂ at a flow rate of 4 ml/ min. As for OPPs the same model of gas chromatograph was used and fitted with a flame photometric detector (FPD) with phosphorus filter. It attached to a fused silica capillary column PAS-1701 (30 min length x 0.32 mm internal diameter x 0.25 μ m film thickness). The Injector and detector temperature program were 240 °C and 250 °C, initial oven temperature, 200 °C for 2 min, raised at 6 °C min⁻¹ then held at 250 °C for 15 min. The carrier gas was nitrogen at 3 ml min⁻¹;

hydrogen and air were used for combustion in 75 and 100 ml min⁻¹, respectively.

4. Extraction and clean- up:

Prior to extraction, all glassware used in this study were washed with soap and water, followed by distilled water and acetone and finally heated at 220 °C in oven till use. The technique adopted to extract and clean-up multi-pesticide residues from milk samples was one used in details by Battu *et al.* (2004).

Recovery analysis was carried out on samples spiked with multi-pesticide residues standards. Extraction and clean- up were carried out on spiked samples as proposed technique. Blank analysis also has been carried out to check the interference in samples. Detected pesticide residues in all examined milk samples were calculated as ppm.

RESULTS

Table 1: Organochlorinepesticide residues (ppm whole milk basis) in examined milk samples.

NO	Detected Pesticides	Incidence		Minimum	Maximum	Mean± SEM	MRLs	
		NO	%				FAO/WHO (2013)	EU (2014)
1	Alpha_HCH	1	3.3	0.00	0.0011	0.00055±0.0005	N.E	0.004
2	gama_HCH	4	13.3	0.00020	0.00300	0.000927±0.00069	N.E	0.001
3	Delta_HCH	25	83.3	0.0006	1.4400	0.182976± 0.07008	N.E	0.003
4	Heptachlor	0.00	0.00	0.00	0.00	0.00	Sum=0.006	Sum=0.004
5	Heptachlorepoxide	28	93.3	0.0002	0.0160	0.00204±0.0005		
6	Aldrin	1	3.3	0.00	0.0001	0.00005± 0.00005	0.006	0.006
7	Gama_chlordan	4	13.3	0.0008	0.0010	0.00095± 0.00005	0.002	0.002
8	Endosulfan	0.00	0.00	0.00	0.00	0.00	0.01	0.05
9	Dieldrin	7	23.3	0.0010	0.0030	0.00186± 0.00034	0.006	0.006
10	Endrin	0.00	0.00	0.00	0.00	0.00	N.E	0.0008
11	PP_DDE	18	60	0.0001	0.0060	0.00143±0.00042	Sum=0.02	Sum=0.04
12	PP_DDD	21	70	0.00010	0.00200	0.00075±0.000092		
13	PP_DDT	0.00	0.00	0.00	0.00	0.00		
14	Methoxychlor	25	83.3	0.01	0.39	0.1424± 0.0244	N.E	0.01

N.E: Not Established

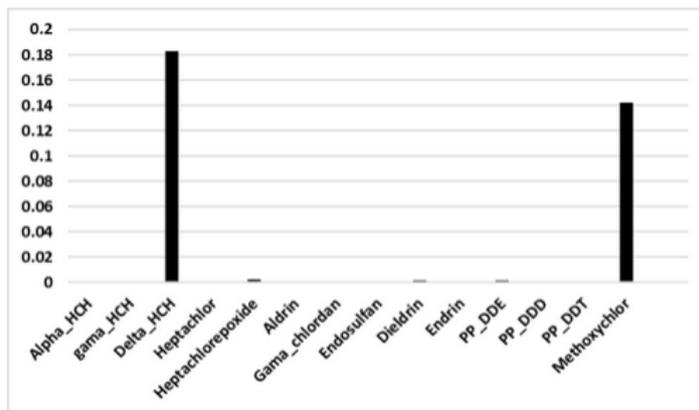


Fig. 1: Mean values of different organochlorine pesticides detected in examined milk samples

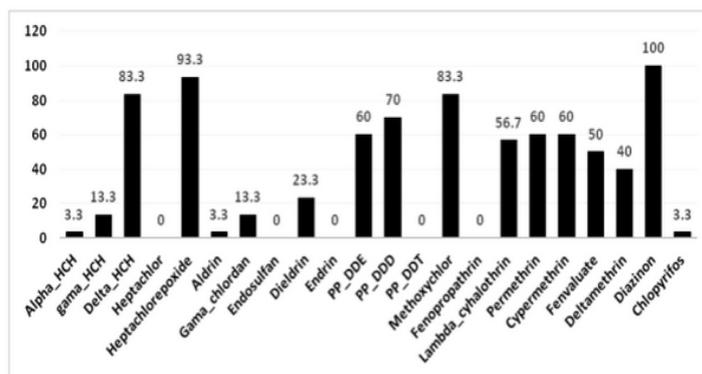


Fig. 2: Incidence of pesticides residues in analyzed milk samples.

Table 2: Pyrethroid pesticide residues (ppm whole milk basis) in examined milk samples.

NO	Detected Pesticides	Incidence		Minimum	Maximum	Mean± SEM	MRLs	
		No	%				FAO/WHO (2013)	EU (2014)
1	Cypermethrin	18	60	0.002	0.050	0.0253±0.0040	0.05	0.05
2	Deltamethrin	12	40	0.01	0.06	0.0417±0.0041	0.05	0.05
3	Fenopropathrin	0.00	0.00	0.00	0.00	0.00	0.01	N.E
4	Fenvaluate	15	50	0.003	0.080	0.0242±0.0065	0.01	N.E
5	Lambda_cyhalothrin	17	56.7	0.002	0.065	0.0156±0.0044	N.E	0.05
6	Permethrin	18	60	0.002	0.060	0.0263±0.0052	N.E	0.05

N.E: Not Established

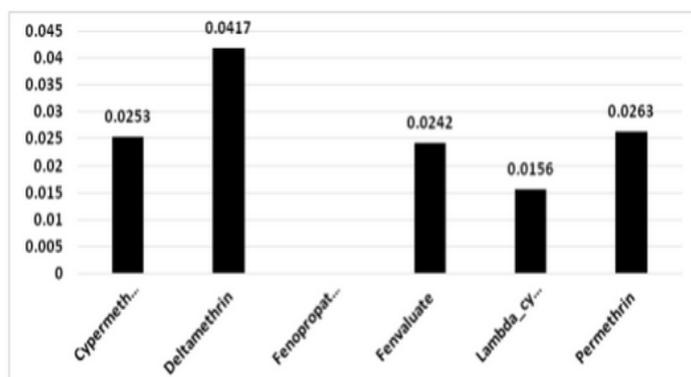
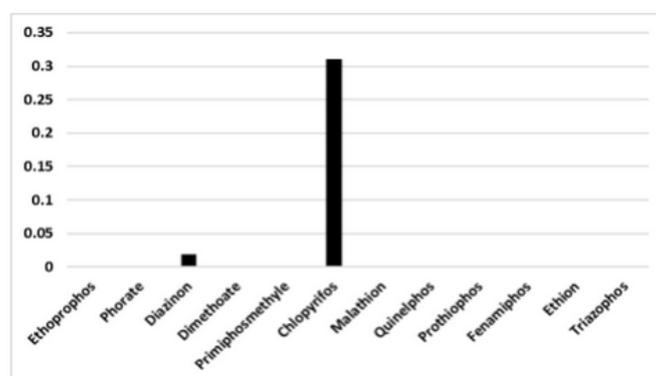


Fig. 3: The mean values of different Pyrethroid pesticides detected in examined milk samples.

Table 3: Organophosphorus pesticide residues (ppm whole milk basis) in examined samples.

NO	Detected Pesticides	Incidence		Minimum	Maximum	Mean± SEM	MRLs	
		No	%				FAO/WHO (2013)	EU (2014)
1	Ethoprophos	0.00	0.00	0.00	0.00	0.00		
2	Phorate	0.00	0.00	0.00	0.00	0.00		
3	Diazinon	30	100	0.01	0.04	0.0183±0.0017	0.02	0.02
4	Dimethoate	0.00	0.00	0.00	0.00	0.00		
5	Primiphosmethyle	0.00	0.00	0.00	0.00	0.00		
6	Chlopyrifos	1	3.3	0.00	0.62	0.3100±0.0017	0.02	0.01
7	Malathion	0.00	0.00	0.00	0.00	0.00		
8	Quinelphos	0.00	0.00	0.00	0.00	0.00		
9	Prothiophos	0.00	0.00	0.00	0.00	0.00		
10	Fenamiphos	0.00	0.00	0.00	0.00	0.00		
11	Ethion	0.00	0.00	0.00	0.00	0.00		
12	Triazophos	0.00	0.00	0.00	0.00	0.00		

**Fig. 4:** The mean values of different organophosphorus pesticides detected in examined milk samples

DISCUSSION

Data in all Tables represented the detected pesticides residues including (OC, OP and Pyrethroids), arithmetic means \pm standard errors and their minimum and maximum residual levels compared with corresponding maximum residue limits (MRLs) recommend by the Codex Alimentarius Commission (FAO/ WHO, 2013) and (European Union (EU), 2014).

Data in Table 1 and Fig 1 reflect that all analyzed raw milk samples were contaminated with OCP residues except heptachlor, Endosulfan, Endrin and DDT. The mostly detected OCP were heptachlor epoxide 93.3 % followed by Delta HCH and Methoxychlor (83.3 % for both) at mean concentrations of 0.00204, 0.182976 and 0.1424 ppm, respectively. pp'-DDE and pp'-DDD was detected 60 and 70 % of the samples. With mean values 0.00143 and 0.00075 ppm, respectively, while pp'-DDT was not detected in the examined milk samples. As for HCH and its metabolites, α -HCH was detected in one sample only with percent (3.3%) while γ - HCH and Delta HCD were detected in 4 and 25 samples with a percent

(13.3 and 83.3 %), While the average concentration were as this follows: α -HCH (0.00055), γ - HCH (0.000927) and Delta HCH (0.182976) ppm. Endrin not detected in examined milk samples while Aldrin and Dieldrin were identified in one sample (3.3%) and 7 samples (23.3 %) at a mean concentrations of 0.0005 and 0.00186 ppm, respectively. γ - chlordan were detected in 4 milk samples (13.3 %) with mean value 0.00095 ppm. Endosulfan was not detected in the examined milk samples. Levels of OCP residues detected (α -HCH, γ - HCH, Delta HCH and Methoxychlor) were above their respective while, other OCP residues blow their respective MRLs recommended by FAO/WHO and European Union legislation.

The technical-grade HCH is a mixture of different isomers (α , β , γ isomers); α -HCH, γ - HCH, Delta HCH were detected in 1, 4, 25 samples at percent 3.3, 13.3 and 83.3 %, while Battu *et al.* (2004) could detect γ -HCH (lindane) in 40.9% liquid milk samples. Abou Donia *et al.* (2010) found γ -HCH in 50% of buffalo's milk samples. The presence of γ -HCH (lindane) in contaminated samples in the present study is consider serious problem, as it is

carcinogenic in nature, and may affect the functioning of other vital organs of the body (Vettorazzi, 1975). Endosulfan is generally assumed to be not passing into milk. So, the most of the monitoring studies of milk and milk products in respect of OCP residues have not reported occurrence of residues of Endosulfan excepting that of Darko and Acquah, (2008) and Nag and Raikwar, (2008) who reported the presence of Endosulfan in dairy products in Ghana and India, respectively.

Higher level of pp' DDE were detected in milk in china (Zhong *et al.*, 2003) who reported that mean concentration of pp' DDE in examined milk samples was 0.038 while lower level of pp' DDE were detected in Ghana (Darko and Acquah, 2008) who reported that mean concentration was 0.001 ppm.

Heptachlor was not detected in the present study, while Salem *et al.* (2009) reported that heptachlor was detected in two milk samples with mean concentration of 0.026 mg/kg fat and its MRL according to (FAO/WHO, 2006) which 0.006 mg/kg fat was exceeded in both samples. John *et al.* (2001) found heptachlor residue in buffalo's milk samples at a level of 0.15 ppm which also exceeded the tolerance limit. Abou Donia *et al.* (2010) detected heptachlor and H. epoxide in 8.3% and 25% buffalo's milk samples with a mean values of 0.032 and 0.064 ppm fat basis respectively, that is exceeded MRLs.

Endrin insecticide is rapidly metabolized to dieldrin in animals, (Smith, 1991). Therefore, the total residues of both endrin and dieldrin insecticides were considered as total dieldrin. The levels of Dieldrin measured in the present study were lower than those reported in a study conducted in Giza, Egypt, in which the mean concentration of Dieldrin in raw milk was 2.966 ± 0.135 mg/kg and the frequency was 55.5% (Ahmed and Zaki, 2009). On the contrary, Salem *et al.* (2009) detected no Dieldrin residues in milk sampled in Jordan. Endrin in the present study was not detected in any examined milk samples due to the limited use of these insecticides.

Organochlorine pesticides pose serious risk to human health and the environment, which may include carcinogenicity, reproductive impairment, developmental and immune system changes and endocrine disruption (IOMC, 2002). The existence of OCP residues in milk with concentrations over than their corresponding MRLs will set off the alarm bell.

Table 2 and Fig 3, showed the presence of pyrethroid pesticide residues in collecting milk samples. Cypermethrin, deltamethrin, Fenvaluate, Lambda_cyhalothrin and Permethrin were monitored in milk samples while, Fenpropathrin was not monitored. The frequency distribution and mean residue levels of these pesticide in milk samples were as follows cypermethrin (60%) 0.0253 ppm, deltamethrin (40%) 0.0417 ppm, Fenvaluate (50%)

0.0242 ppm, Lambda_cyhalothrin (56.7%) 0.0156 ppm and permethrin (60%) 0.0263 ppm. Fenpropathrin were not detected in all examined milk samples.

The cypermethrin concentrations that found in milk samples were not exceeded the limits of FAO/WHO and EU. Increase concentration of Cypermethrin may cause adverse health impacts While, residue levels of permethrin were above these limits.

Currently, synthetic pyrethroids are extensively and frequently used because they are effective against different pests in agriculture, livestock and public health purposes. They have selective toxicity towards insects as well as their low toxicity to mammals and animals comparing with OCPs and OPPs (Kidd and James, 1991). Cypermethrin, is classified as restricted pesticide by EPA because its toxicity to fish, but in Egypt, it is still used to control a broad spectrum of pests in agriculture and veterinary sectors. Also, it is used to Pesticides control the pests at home and buildings (Extoxnet, 1996).

Table 3 revealed that the mean concentration of Diazinon and Chlopyrifos were 0.0183 and 0.310 mg/kg in milk samples with incidence rate 100 and 3.3 %, respectively. While, other organophosphorus insecticide such Malathion, phorate Ethion, dimethoate, primiphosmethyl were not detected. Our results were disagreeing with Fagnani *et al.* (2011). Who detected malathion in milk at a mean concentration of 0.02 mg/kg and a positive frequency of 8.33%. Also, disagree with (Shaker and Elsharkawy, 2015). Who they detect that the mean concentration of Malathion in the analyzed milk samples was 0.195 mg/kg. The main sources of pesticide residues in milk samples are contaminated feed, grass or corn silage, and direct application of pesticides on dairy cattle in barns or dairy plants. (Johansen *et al.*, 2004).

CONCLUSION AND RECOMMENDATION

From the obtained results, it could be concluded that OC, Pyrethroid, and OP residues were detected in examined raw milk samples. Some detected values of pesticides residues were exceeded the maximum residues limits of FAO/WHO and EU. The risk of using pesticides especially OC pesticides on human health was associated with cancers and increased risk of Alzheimer disease (Singh *et al.*, 2013), so the main management tool is the prevention of exposure either through feed or from animal environment as well as application of withdrawal time (Kan and Meijer, 2007), and monitoring the pesticides residues continuously in milk is very essential to safeguard the consumer health and achieve the food safety.

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الكشف عن بعض متبقيات المبيدات الحشرية فى اللبن الخام

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نظرا لكثرة استخدام للمبيدات الحشرية فى الاونة الاخيرة فى مقاومة الافات الزراعية والتفيليات الخارجية الناقلة للامراض المختلفة الى النباتات والحيوانات والانسان ، الامر الذى أدى الى تلوث الالبان بمتبقيات المبيدات الحشرية التى لها خطورة شديدة على صحة الانسان لما قد تسببه من التسمم بالمبيدات وإحداث طفرات خلقية وجينية وسرطانات. لذلك، أجريت تلك الدراسة على ٣٠ عينة لبن مجمعة عشوائيا من محافظة البحيرة وذلك لتحديد تركيز المتبقيات العضوية الكلورونية ، ومتبقيات البيريثرويد المصنعة و المتبقيات العضوية الفسفورية. وكشفت النتائج عن وجود ألفا وجاما و دلتا هكساكلوروسيكلو هكسان، والايوكسيد سباعي الكلور، ألدرين، جاما- الكلوريدان، الديلدرين، دى دى إى ، دى دى دى ، ميثوكسيكلور بنسب ٣.٣، ١٣.٣، ٨٣.٣، ٩٣.٣، ٣.٣، ١٣.٣، ٢٣.٣، ٦٠، ٧٠، و ٨٣.٣ % فى عينات اللبن التى تم فحصها بينما كانت العينات خالية من سباعي الكلور، الاندوسولفان، الإيندرين و دى دى تى. أما فيما يتعلق البيريثرويدات المصنعة (سايبيرمثرين، دالتامثرين، فينفياليتي، لامبدا_سيهالوثرينوبيرميثرين) فقد وجدت بنسب ٦٠ و ٤٠ و ٥٠ و ٥٦.٧ و ٦٠ % من العينات التى تم فحصها على التوالي فى حين أنفينوبروباثرين لم يكن موجودا فى عينات اللبن. وفيما يتعلق المبيدات الفسفورية فقد كانت جميع العينات ملوثة بالديازينون ٣٠ (١٠٠٪)، ولم يوجد الكلوربيريفوس إلا فى عينة واحدة فقط ١ (٣.٣٪)، وكانت العينات خالية من باقى الانواع الاخرى. ونظرا لمخاطر التعرض لبقايا المبيدات التى تهدد صحة المستهلك فى مصر. فإنه لابد من الكشف عن متبقيات المبيدات الحشرية فى الحليب لحماية صحة المستهلك.