DETECTION OF SOME INSECTICIDE RESIDUES IN MILK AND INFLUENCE OF HEAT TREATMENTS AND BACTERIAL FERMENTATION AGAINST THESE INSECTICIDES

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ABSTRACT

Sixty buffalo's raw milk samples were analyzed for detection of insecticides residues in Port Said governorate. The analysis revealed that presence of organochlorine compounds (DDT, DDE, DDD, Lindan and Dieldrin in 46.67%, 48.33%, 38.33%, 45% and 30% of samples respectively .while organophosphorous compounds could be detected in 21.66%, 11.66%and 8.33% for Diazinon, Malathione and Dursban respectively. None of the milk samples exceeded the permissible limit. Heat treatment and Bacterial fermentation for some positive samples revealed reduction in residual percent (DDT, DDE, Diazinon, Malathion and Dursban) .In contrast, residues of DDD were showed slightly increased by heat treatment, while Lindan and Dieldrin were relatively constant by heat treatment and Bacterial fermentation. The public health importance of insecticide residues in milk and the recommendation for eliminating the milk contamination and safeguard the consumers against the serious effect of these pollutants were discussed.

INTRODUCTION

Milk is an essential food of high nutritive value specially for infants and the aged persons. Milk considered as a cheap source of nearly complete food since its good source not only for protein but also fats, major minerals needs and essential nutrients that the body needs as calcium,protein,potassium and some other vitamin ;A, B2, B12,Niacin and phosphorous, ***(Kholif et al.,1994 and Abou-Arab 1996).***

Milk is considered as a source of excretion of some toxic compounds (organochlorine compounds) which are excreted by simple diffusion Both basic substances and lipid-soluble compounds can be excreted into milk, where basic substances can be concentrated in milk since milk is more acidic (Ph=6.3-6.5) than blood plasma (PH=7.3-7.5) ***(Ebbing and Wrighton 1987).***

The contamination of milk is considered as one of the main dangerous aspects in the last few years .Milk can be contaminated by residues of organochlorine and organophosphorous through a variety of sources ***(Abou-Arab1996 and Fontcuberta et al., 2008.)*** Insecticides reach milk through exposure of dairy animals to direct spraying with insecticides used for controlling of external parasites on animals or via polluted food and soil ***(Snelson and Tuinstra 1979 and Waliszewski et al.,1997).***

Organochlorine pesticides (OC) were widely used world wide until restrictions were introduced in the late seventies both in Europe and the USA, initially for DDT ***(Fontcuberta et al., 2008)***.Some of these pesticides are still wildly used by farmers because of their effectiveness and their broad-spectrum activity ***(Amoah et al., 2006)*** and also are being extensively used in tropical countries in malaria control programs and against livestock ectoparasites and agricultural pests ***(Curus 1994).*** Persistent OC compounds such as DDT andHCB play an important role in chronic poisoning and take part in a number of pathological processes ***(Lembowiez et al., 1991 Sitarska et al., 1991).***

Fat solubility of these compounds is responsible for their varied concentrations in the tissues and their accumulation in the lipoproteins of the cell membranes, thus changing their structures and permeability ***(Chowdhury et al. 1990 and Antunes et al. 1993).*** Organochlorine pesticides are able to significantly decrease the ability of highly purified human natural killer (N K) cells to lyse tumor cells after exposure, ranging from 1 hour to 6 days ***(Beach and Whalen2006).*** Pesticide exposure independently or in synergism with modifiable risk factors, is recognized as an important environmental risk factor associated with hemopoetic cancers, cancers of the prostate, pancrease, liver and other body systems ***(Jaga and Dharmani, 2005).***

However the use of most OC pesticides in Egypt was restricted. Nevertheless, they are present in the environment. The fermented dairy products such as yoghurt are also important. Since their beneficial effects make them a valuable component in the diet of infants and elderly .Dairy products can be contaminated by pesticides by improper handling and by feeding the 3/1/2017animals on contaminated feeds. Many researches have demonstrated that organochlorine concentrations become considerably reduced in the course of food processing. The removal of residues is influenced by the type of food, location of pesticide and especially by the type of processing operation ***(Abou -Arab 1997).***

This study was performed to detect the presence of some organochlorine and organophosphrous insecticides in buffalo's milk samples and to evaluate the influence of heat treatments (pasteurization, boiling, sterilization and bacterial fermentation) on the level insecticides residues in milk.

MATERIAL AND METHODS

Materials:

Milk: A total of 60 buffaloes' raw milk samples (1 litre of each) randomly collected from different areas in Port-Said Governorate and its surrounding areas over a period of summer months of insecticides application in agriculture and veterinary purposes. Each sample was received in a glass bottle and immediately cooled in an ice box and then quickly stored at -20°C until analysis. Before analysis, the milk samples were shaken handly and subjected directly to extraction process.

Methods of Analysis:

Insecticide residues were extracted from different samples and analyzed according the method described by ***(Toyoda et al., 1990, Pesticide Analytical Manual 1991 and A.O.A.C 1995)*** using Hewlett Packard Model 5890 G.L.C.equiped with electron Ni63 and flame photometric Detector.

Heat treatments:

Raw positive samples of buffaloes milk (5% fat) were exposed to each of the following treatments:

1- **Pasteurization:** 7positive milk samples were heated to73ºC for 15 sec .and cooled to 10ºC (Laboratory Pasteurization).

2- **Sterilization:** 7positive milk samples were sterilized in an autoclave, at 121ºC for 5 min.

3- **Boiling:** 7positive milk samples were heated with stirring to boiling point for five minutes then cooled.

4- **Yoghurt Processing:** 7 positive milk samples were heated to 80-82 ºC in a water bath for 20minutes, and cooled to 40-42 ºC.Milk were inoculated with 2%yoghurt starter culture (1:1) mixture of *Streptococcus thermophilus and Lactobacillus bulgaricus* at 40ºC for3-4 hours until it was coagulated and then refrigerated overnights as described by Egyptian Organization for Standardization ***(E.O.S. 1970).***

After heat treatment and bacterial fermentation of (7) positive samples. Each sample was analyzed for insecticide residues. Achieved data were statistically analyzed.

RESULTS AND DISCUSSION

1- <u>Insecticide residues in milk:</u>

The analysis of 60 samples of raw milk as shown in Table (1) revealed that the organochlorine insecticides residues were detected at percentage of 46.67%, 48.33%, 38.33, 45% and 30% for p.p.DDT, p.p.DDE, p.p.DDD, Lindan and Dieldrin respectively. The concentration of p.p.DDT were ranged from 0.045 to 0.196 µg/L with a mean value of 0.090 ± 0.020 The p.p.DDE varied from 0.080 to 0.197µg/Lwith an average value of 0.135 ± 0.009.Also, the concentration of p.p.DDD were ranged from 0.033 to 0.206 µg/L with a mean value of 0.099 ± 0.015 .Moreover the average value of Lindan was 0.039 ± 0.010 and its quantities lie between 0.011 to0.059 µg/L but the mean value of Dieldrin was 0.092 ±0.027 and its residues varied from 0.060 to 0.165 µg/L .Aldrin couldn't be detected in raw milk samples .These results are nearly similar to those reported by ***Ali et al., (1993), Samia (1997)and Nevin and Eman(2009).*** However, none of the detected organochlorine residues exceed the permissible limit. In this respect the average levels are below the acceptable maximum residues level (MRL) mentioned by ***F.A.O and WHO (1972).*** These limits are 1.25 P.P.m. for total DDT and its isomers (DDD, DDE) , 0.020 P.P.m .for Lindan and 0.15 P.P.m. for Dieldrin and Aldrin . In contrast ***Kannan et al., (1992) and Amr et al., (1996)*** recorded that some of organochlorine compounds in milk were found to be above the MRL of FAO and WHO. On the other hand DDT was the highest residue concentration in raw milk. **Battu et al. (2004)** revealed that DDT residues were exceeding the MRL in 74% of samples of liquid milk in India. In this aspect, ***Waliszewski et al. (2004)*** demonstrated that DDT is an insecticide that has been widely used because of its chemical stability. DDT is Known to be degrade into DDD, DDE and certain other metabolites in the environment and such compounds are lipid soluble and bioaccumulate. They added that DDE is the major metabolite and may account for about 25% or more of the total DDT. Further more, the frequent detection of this organochlorine in raw milk fat showed that their persistance in the environment is due to their use in agriculture, volatilization from contaminated soils and thus contaminated growing plants.

The present results recorded that Aldrin residues was not detected in all examined milk samples which may be due to continuous degradation into Dieldrin within living tissues through different metabolic processes ***(Dogheim et al., 1989 and Samia1997).***

Regarding to the analytical result of organophosphorous compounds as shown in Table (1) Diazinon residues could be detected in 21.66% of examined milk samples ranging from 5.33 to 184.00 µg/L with a mean value of 130.120 ± 24.6 such level is considered slightly high and might be due to large scale of application of Diazinon for the control of external parasites during this period of the year. These results are nearly similar with those reported by ***Pagliuca et al., (2006).*** In contrast ***Toyoda et al., (1990)*** and ***Kholif et al., (1994)*** recorded higher concentration of Diazinon residues in milk samples. On contrary ***Abou-Donia et al., (2010)*** recorded that none of the buffalo's milk samples revealed the presence of an organophosphorous residue. However the ***food and Agriculture organization*** and ***world Health organization (1972)***, recorded that the acceptable daily intake for Diazinon residues in milk was been set at a maximum of 2µg/Kg body weight. Analytic results revealed that Malathione residues in milk samples were 11.66 and its residues were varied from 0.048 to 0.357 ug/L with a mean value of 0.107± 0.036.These values are considered lower than those reported by ***Abd-Alla et al., (1991) and Samia (1997)***. Results revealed the presence of Dursban in 8.33% of milk samples with a mean concentration value of 0.056 ± 0.016 ug/L. such results are in agreement with those reported by ***El-Ghannam et al.,(1987)***who detected Dursban residues in milk after spraying stayed for beyond the toxic level for human. ***WHO(1973)*** reported that the level of 0.03 mg/Kg body weight per day appear to be the minimal response level in human to Dursban,while the tolerance of 0.014 mg/Kg body weight per day orally for one month is a level causing no harmful effect. Table (1) demonstrated that raw milk samples were free from detectable amounts of Lannte, Dimethoate, Parathion and Phosalone. On the other ***hand Stijve (1984), Camoni et al., (1990), Abd-Alla et al., (1991)*** and ***Pagliuca et al., (2006)*** reported deposition of these residues and other pollutants in milk.

The present study revealed that organochlorine residues in milk samples are comparatively higher in comparison with organophosphorous residues. In this aspect ***Downey (1971)*** explained that organophosphorous insecticides are rapidly decomposed by physicochemical processes in the environment as well as by enzymatic processes in the animal body which prevents the building up of significant residues in milk. In addition ***I.D.F. (1979)*** reported that most of these compounds are water soluble, animals secrete most of them in urine and faeces so little or no residues would be secreted in milk**.**

Moreover,organochlorine are very stable compounds resulted in extensive environmental pollution, have high fat and water accumulated in adipose tissue of milking animals , then high residues would be secreted in milk. The most dangerous problem of milk pollution with insecticides, is that it causes changes in milk content which give chance to be teratogenic substances to human consumption and cause a generation of unexpected diseases .Since some insecticides accumulate in foetal tissues indicating placental transfer of such chemicals, in addition to renal failure, liver cirrhosis and optic never manifestation, ***(Dixon 1980 and Amr 1992).***

However, it can be generally stated that the contamination of milk by OCPS and PCBS (polychlorinated biphenyls) has declined in developed countries as a result of the restrictive measures in pesticide usage applied by governments following the recommendation of the ***World Health Organization (WHO) and the Food and Agriculture Organization (FAO).***

Table (1): Insecticides residues of organochlorine and organophosphorous in buffalo's raw milk samples (µg/litre).

Type of Insecticides	No. of Samples	No. of positive	٪	Range of concentration µg /Liter		Mean ± S.E	Permissible limit of FAO/WHO	
				Minimum	Maximum		daily intake limit µg/60Kg B.W.	limit in milk P.P.m
Organochlorine								
P.P. DDT	60	28	46.67%	0.045	0.196	0.090 ± 0.020		1.250
P.P. DDE	60	29	18.33%	0.080	0.197	0.135 ± 0.009	1200.0	
P.P. DDD	60	23	38.33%	0.033	0.206	0.099 ± 0.015		0.020
Lindan	60	27	45%	0.011	0.059	0.039 ± 0.010	600.0	
Dieldrin	60	18	30%	0.060	0.165	0.092 ± 0.027	6.0	0.150
Aldrin	60	N.D	N.D	N.D	N.D	N.D		
Organophorous								
Diazinon	60	13	21.66%	5.330	184.000	130.12 ± 24.6	0.2	
Malathione	60	7	11.66%	0.048	0.357	0.107 ± 0.036		
Dursban	60	5	8.33%	0.098	0.348	0.056 ± 0.016	30.0	
Lannate	60	N.D	N.D	N.D	N.D	N.D		
Dimethoate	60	N.D	N.D	N.D	N.D	N.D		
Parathion	60	N.D	N.D	N.D	N.D	N.D		
Phosalone	60	N.D	N.D	N.D	N.D	N.D		

2- Effect of Pasteurization, Boiling and Sterilization on organochlorine residues in milk:

The effect of heat treatment on the concentration of insecticides in buffalo's milk was illustrated in Table (2).The results revealed that pasteurization process causing reduction in each of p.p.DDT and p.p.DDE residues in a level of 26.47% and 9.52% respectively., while boiling process causing reduction by 11.76% and 7.14% in p.p.DDT and p.p.DDE respectively. These results are in accordance with that recorded by ***Abou- Donia et al., (2010).***

On the other hand pasteurization and boiling causing increase by percentages of 22.80% and 18.42% in p.p.DDD residues. Such results are inagreement with those reported by ***Abdrabo et al. (1989), Amr et al. (1996), Samia (1997) and Nevin and Eman (2009).*** The increase of p.p.DDD level of may be due to decomposing of p.p.DDT and p.p.DDE during thermal treatment into p.p.DDD as reported by ***Dick et al., (1978)*** and ***Heschen et al., (1978).***

Our results showed that Lindane and Dieldrin residues tend to be thermostable against pasteurization and boiling process. Slight reduction could be detected in linden and Aldrin by ***Abou-Donia et al., (2010).***

Regarding to Table (2) sterilization process cause reduction in organo- chlorine residues of milk samples in a ratio of 29.41%, 26.16% on DDT and DDE respectively, while the ratio of DDD residues was increase to11.40%by sterilization. These results are in agreement with those reported by ***Abou-Arab (1991) and Abou-Donia et al., (2010).*** The mean concentrations of organochlorine pesticides in raw, pasteurized and UHT milk samples are reported by ***Heck et al., (2007)*** who found that

One-Way ANOVA revealed that p.p.DDD and total DDT levels were significantly higher in raw milk than in pasteurized and UHT milk ,while p.p.DDDwas significantly higher in UHT than in raw milk.

However organocholorine pesticides and their residues are highly lipophilic nature and are hardly metabolized (***Falandysz et al., 2004)***, they it may easily concentrate in fatty foods such as milk products leading to bioconcentration and biomagnifications through food chain ***(Darko and Acquaah, 2008).***

3- Effect of pasteurization, Boiling and sterilization on organo-phosphorous residues in milk:

The result tabulated in Table (2) revealed that each of pasteurization, boiling and sterilization had a noticable effect in degradation of Diazinon, Malathion and Dursban residues where the reduction percentages as result of pasteurization were 7.677%, 46.97% and 42.85% respectively while the data were 23.26%, 56.59% and 57.14% due to boiling process and 40.162%, 59.44% and 72.34% for Diazinon, Malathion and Dursban respectively due to sterilization process. These results are nearly in agreement to those reported by ***Fekry*** and ***Bahout (1992)*** and ***Samia (1997).*** However, ***Korolev (1987)*** investigated the efficiency of heating the milk to 40 , 100 or 126 ºc in water bath for 15 or 30 minutes for decontamination of milk from Methatim and other organophosphorous insecticides. He concluded that thermal treatment at 100 ºc and 126 ºc were effective in destruction of all examined organophosphorus insecticides residues at percentages of 93.3% and 100% respectively with exception of sebacyl which showed only 34-40% destruction.

Table (2): Effect of heat treatment (Pasteurization, Boiling and Sterilization). On the level of insecticide residues in buffalo's raw milk samples.

Type of heat treatment	Raw milk	Pasteurized milk		Boiling milk		Sterilized milk	
Type of Insecticides	Mean ± S.E	Mean ± S.E	degradation %	Mean ± S.E	degradation %	Mean ± S.E	degradation %
Organochlorine							
P.P. DDT	0.034 ± 0.053	0.025 ± 0.051	26.47	0.030 ± 0.044	11.76	0.024 ± 0.06	29.41
P.P. DDE	0.042 ± 0.037	0.030 ± 0.026	9.52	0.039 ± 0.099	7.14	0.031± 0.077	26.16
P.P. DDD	0.114 ± 0.066	0.140 ± 0.078	22.80	0.135 ± 0.098	18.42	0.127 ± 0.087	11.40
Lindan	0.0125 ± 0.059	0.0123 ± 0.057	Thermostabl or no change	0.0123 ± 0.057	No change	0.0124 ± 0.056	No change
Dieldrin	0.090 ± 0.024	0.091 ± 0.023		0.090 ± 0.024		0.090 ± 0.024	
Organophorous							
Diazinon	130.12 ± 24.60	120.130 ± 11.21	7.677	99.850 ± 9.66	23.26	77.860 ± 7.55	40.162
Malathione	0.364 ± 0.099	0.193 ± 0.065	46.970	0.158 ± 0.086	56.59	0.146 ± 0.067	59.44
Dursban	0.049 ± 0.042	0.028 ± 0.08	42.850	0.021 ± 0.007	57.14	0.013 ± 0.005	72.34

4- Effect of yoghurt processing on the level of insecticides residues in milk:

The fermented dairy products such as yoghurt are also important since their beneficial effects make them available components in the diet of infant, children and Elderly. The results tabulated in Table (3) revealed that processing of milk into yoghurt reduced the level of organochlorine insecticides residues at percentages of 44.11%, 57.14%, 5.26%36% and 25.55% for DDT, DDE, DDD, Lindan and Dieldrin respectively. These results are nearly similar to those reported by ***Abou-Arab (1991), Samia (1997), Darko and Acquaah (2008)*** and ***Abou-Donia et al., (2010)*** which show that the manufacture of yoghurt from buffalo's milk result in reducing the level of studied pesticides such as Lindan, Aldrin, DDT, DDE, and DDD. The reduction may be due to the heat treatment of milk 80-82 ºc for 20 min. and as the activity of yoghurt starter culture *(S.thermophillus* and *L. bulgricus).*Yoghurt processing reduced the organophosphorous residues in milk by 9.25%,47.80% and63.26% for Diazinon, Malathione and Dursban respectively .It may be attributed to milk protein adsorption, bacterial cell incorporation and or microbial degradation ***Magdoub et al.,(1989)***These results are in coordinated with those reported by ***Gajduskova and Lat(1974)***.In this aspect, ***Abou-Arab (1997)*** reported that milk processing technologies have an important role in reduction of insecticide residuesin milk products and supplying safe food for human than raw milk. Furthermore, the residues contents in dairy products reflect their regional environmental contamination and may be of great value for scientific and public health knowledge.

The present result can conclude that heat treatment more effective in degradation of insecticides, in addition to the manufacture of yoghurt decreased the level of studied insecticides. Since the consumption of milk products may be safer than raw milk so we recommended that:

1- It is preferred that milk contaminated with insecticides under go industrial manufacture instead of consumption of milk as raw milk.

2- Regulations and control using of these insecticides to minimize or eliminate the hazard resulting from miss use of these compounds.

Table (3): Effect of yoghurt processing (Bacterial fermentation). On the level of insecticide residues in buffalo's raw milk samples.

Type of Insecticides	Raw milk	yoghurt	
	Mean ± S.E	Mean ± S.E	degradation%
Organochlorine			
P.P. DDT	0.034 ± 0.053	0.019 ± 0.082	44.11
P.P. DDE	0.042 ± 0.037	0.018 ± 0.081	57.14
P.P. DDD	0.114 ± 0.066	0.120 ± 0.75	5.26
Lindan	0.0125 ± 0.059	0.017 ± 0.004	36
Dieldrin	0.090 ± 0.024	0.067 ± 0.018	25.55
Organophorous			
Diazinon	130.12± 24.60	118.08 ± 20.59	9.25
Malathione	0.364 ± 0.099	0.19 ± 0.040	47.80
Dursban	0.049 ± 0.042	0.018 ± 0.05	63.26

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الكشف عن وجود بعض المبيدات الحشرية في الألبان وتأثير المعاملات الحرارية والتخمر البكتيري ضد هذه المبيدات

أجري هذا البحث للكشف عن وجود بقايا بعض المبيدات الحشرية في الألبان. وقد تم تحليل (٦٠) ستون عينة من اللبن الجاموسي فى محافظة بورسعيد. وقد أسفرت النتائج عن وجود مبيدات DDTو DDE و DDD و Lindan و Dildrin بالنسب الآتية على التوالي: ٤٦,٦٧٪ ، ٤٨,٣٣٪ ، ٣٨,٣٣٪ ، ٤٥٪ ، ٣٠٪. وهم من مركبات الهيدروكلورين، بينما كانت نسبة المركبات الفسفورية ٢١,٦٦٪ ، ١١,٦٦٪ ، ٨,٣٣٪ ، لكل من الديازينون والملاثيون والدورسبان على التوالي . ولم تتعدى كل من النسب الناتجة للمركبات، النسب المسموح بها. أما تأثير المعاملات الحرارية المختلفة وتصنيع اللبن إلى زبادي على هذه المتبقيات فقد أظهرت النتائج تأثيراً ايجابيا في تكسير متبقيات المبيدات الحشرية في اللبن ، فقد اختزلت النسب الناتجة في المركبات الهيدروكلورونيه (عدا نسبة DDD التي زادت بعض الشئ عن النسبة قبل المعالجة الحرارية) والمركبات الفسفورية وقد نوقشت الأهمية الصحية لمتبقيات المبيدات الحشرية في اللبن والاحتياطات الواجب اتخاذها أو التخلص من تلوث الألبان بهذه المبيدات وحماية المستهلك ضد هذا التأثير الخطير لهذه الملوثات.