Determination of insecticide Deltamethrin residues in local and imported raw milk samples collected from different animal's species and the effect of processing heat treatment on its content in milk

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Summary

A total of 163 milk samples (500 ml each) of cows, ewe, goats, buffaloes, camels were collected randomly at weekly intervals (10 samples/ week) from both Abu-Ghraib and Al-Fudhailiya villages and also from different local retail markets inside Baghdad province. These samples include milk of different animal species, milk cans, bulk milk tanks and imported Ultra-high temperature processing milk. Among the total milk samples only 138 milk samples were examined during two climatic periods where the first period was in summer which extended from the beginning of September to the end of October 2015 while the second period was in winter which extended from the beginning of January to the end of February 2016. Besides, some of the selected positive samples for Deltametrin residues were subjected to one of the commercial heat treatments such as 63°C/30 min., 80°C/5 min. and 100°C/5 min. to evaluate the efficiency of heat exposure on the degradation of Deltametrin residues in milk. The results pointed out that milk samples containing the higher fat percentage exhibited significantly ($P<0.05$) a higher concentration of Deltametrin in summer (0.08ppm) than in winter (0.008 ppm). It was clearly obvious that the detectable concentrations of the Deltametrin were higher in buffalo's and ewe milk samples than those found in cows, goats and camels and such results could be attributed to the higher fat content of buffalos and ewes milk than the other animals as well as the lipophilic nature of the Deltamethrin. In other word, increase of fat percentages of milk was associated with an increased level of Deltametrin residues due to the lipophilic nature of the Deltametrin pesticide. The current results revealed that milk samples that were collected from buffaloes, ewes and cows recorded significantly ($P<0.05$) the highest Deltametrin residues in summer season whose mean levels exceeded the accepted MRLs of 0.05 ppm to milk samples of goats and camels that had significantly ($P<0.05$) the lowest mean levels of Deltametrin residues whose means levels were 0.038 and 0.032 ppm respectively. There was a significant ($P<0.05$) seasonal variation of the Deltametrin concentrations in milk samples for each animal species where all the milk samples that were collected from buffaloes, ewe, cows, goats and camels had significantly ($P<0.05$) higher mean levels of Deltametrin residues in summer season than in winter season. Milk samples that were collected from milk cans (5, 25 and 50 kg) recorded significantly ($P<0.05$). The highest Deltametrin residues during the summer season in comparison to 10 tons bulk milk tank samples.

Keywords: High perform liquid chromatography, Deltamethrin, Whole milk, Skimmed milk.

Introduction

Deltamethrin (DMT) is Type II synthetic pyrethroid pesticides produced in 1974 and first marketed in 1977(1). Deltamethrin products are among some of the most popular and widely used insecticides in the world in competing ectoparasites affecting farm animals (2). The contamination of milk with pesticides is considered as one of the main dangerous aspects in the last few years. Milk has been found to be very good reference point for monitoring contamination by pesticides (3). A considerable portion of such pesticide residue may penetrate the skin through the blood and appear in the milk and meat (4). Transfers of such pesticide to our body via food of animal origin rich with fat contents such as milk has not been studied till now in Iraq and since there are no data on the residual levels of Deltamethrin in different animal and milk product routes of exposure in Iraq, this study was conducted to determine the levels of

Deltamethrin pesticide residues in cows, ewe, buffaloes, goats, and camels milk samples collected from Baghdad province, using high performance liquid chromatography (HPLC). The aims of the current study were to determine the levels of Deltamethrin residues in raw cows, ewe, goat's, buffaloes, camels milk samples collected from different areas in Baghdad province, measure the fat content of milk samples to ascertain whether higher residue levels found in milk samples are related to higher fat content, and to assess the effect of heat treatment (pasteurization) on availability of Deltamethrine residues in raw milk.

Materials and Methods

This study was carried out during the period extended from the beginning of September 2015 to the end of the February 2016. A total of 163 milk samples (500 ml each) were collected randomly at weekly intervals (10 samples/ week) in sterile polyethylene plastic bags from both the Abu-Ghraib and AL-Fudhailya villages and from different local super markets inside Baghdad province. All the collected milk samples were transported to the laboratory of veterinary public health department at the college of veterinary medicine inside portable ice-cooled containers, 138 milk samples were examined on two climatic periods, the first was the summer period that extended from the beginning of September to the end of October 2015 while the second was the winter period that extended from the beginning of January to the end of February 2016. Each milk sample was divided into two portions where the first portion (300 ml) was used to determine the chemical composition of milk such as the specific gravity and the percentages of fat, lactose, protein, solid non fat and minerals by using the ultrasonic milk analyzer. While the second portion of (200 ml) was sent to the HPLC analysis to determine the level of deltamethrine residues, besides that some of selected positive samples for deltamethrine residues were subjected to one of the industrial heat treatments such as 63°C/ 30 min. 80°C/ 5min. and 100°C/ 5 min. to determine the effect of heat treatment on the levels of Deltamethrime residues in milk.

Milk sample extraction and separation procedure for DMT qualification in milk samples: 200 ml of milk sample were taken in a conical flask, then 10 gm of zinc acetate were added to the milk to remove the fats and the milk filtrate was added in a separating funnel. To extract DMT from this extract, 150 ml of n-hexane were added in a separating funnel. After shaking of n- hexane with the milk sample for 4 min., the organic layer containing the DMT was separated and further 50 ml of n-hexane were added to the aqueous layer to remove the possible traces of DMT. The filtrate (organic layer) was transferred to 250 ml separating funnel and partitioned by 100 ml acetonitrile and the above mixture was shaked for 3 minutes and acetonitrile layer was collected separately. 10 gm of anhydrous Na_2SO_4 were added to the acetonitrile layer to remove the water contents, at the end, the DMT extract was cleaned by passing on silica column (200×5 mm I.D) using 50 ml of n-hexane followed by 50 ml ether as mobile phase, the DMT fraction was subjected to stream of nitrogen to evaporate the solvent and the final residues were dissolved in 2 ml of the separating mobile phase. The extract was filtered on disposable minister filters 0.2 um (supelco company cat No 16534K) then 20 ul were injected on HPLC column. The concentration for each compound was quantitatively determined by comparison the peak of area of the standard with that of the samples (5).

HPLC condition: The extracts were separated on FLC (Fast liquid chromatography) columns: C-18DB, 2.7 µm particle size (50×4.6 mm ID). Mobile phase: 0.1% acetic acid in deionized water (solvent A) and acetonitrile (solvent B) (20:80, v/v). Detection: UV spectrophotometer detector set as 275 nm. Flow rate: 1.5 ml/ min. Temperature: 30 ˚C. Data submitted to statical analysis by using SPSS. One-way (ANOVA) and least significant differences (LSD) were used to differentiation among means (6).

Results and Discussion

Comparison of the mean levels of DMT residues between the full fat and skimmed milk samples: Results illustrated in (Table, 1) revealed that in raw milk and skimmed milk

with fat from 3.25% to 1.09% the DMT residues ranges from 0.104 and 0.00 ppm. The current results established the statistically significant (P<0.05) influence of the fat percentage on the concentration of the DMT residues in milk samples. In the USA, Australia and Canada the skim milk is made when all the milk fat is removed from the whole milk by using a separator (7). The results of the current study were in agreement with (8), who reported that skimming of buffaloes and cows milk lead to a slight reduction in the analyzed pesticide concentration in skim milk. Also the results were in agreement with (9) who found that skimming of whole milk led to slightly reduction of BHC, lindane and DDT in milk. Also (10) reported that some pesticides were removed with skim milk and butter milk due to the associated of residues with the phospholipids in milk fat. (8) Reported higher reduction levels in lindane and DDT by skimming. Difference in the reduction levels of pesticides could be attributed to the skimming process and the higher losses of fat in the skim milk.

The effect of different industrial heat treatments on the degradation of the DMT residues (ppm) in cows milk. Exposure of raw whole milk samples to one of the industrial heat treatments such as 63 ºC/30 min., 80 ºC/5 min., and 100 ºC/5 min. caused a significant (P<0.05) reduction in the concentrations of DMT residues at the rate of 15%, 35% and 78%, respectively (Table, 2).

Boiling of the raw whole milk samples for 5 min. offered an effective approach since it greatly reduced the levels DMT residues to less than the MRLs that recommended by the USA and European legislations. The degradation of the DMT residues in milk depends on both the time and degree of temperature treatment. The works (11) revealed that high temperature food processing lead to large reduction in pesticide levels in milk product. DMT was decreased in pasturised raw milk samples and pasteurization was proved to be the least effective on the degradation or elimination of pesticide residues (12). Pasteurization removed malathion completely and reduced dimethoate level to a great extent where the degradation of dimethoate was 73.42% while malathion was completely degraded 100% and not detected in milk samples, sterilization process on the other hand, had a significant effect on both the dimethoate and malathion contents (13).

Table, 1: Comparison of the mean levels of Deltamethrin residues (ppm) between the cow's raw whole and skimmed milk samples collected from the Abu-Ghraib dairy plant.

Source of samples	No. of examined samples	Fat% Mean	DMT (ppm) Mean ±S.E	%violation MRLs 0.05
Whole milk (full-fat)	5	3.256	0.104±0.22 A	100%
Skimmed milk (low fat)	5	1.090	0.0±0.0 B	NV*

LSD= 4.7. Different capital letters in a column revealed significant (P<0.05) differences in the mean levels of Deltamethrin residues (ppm) between the full fat and skimmed milk samples.
NV: No violation.

Table, 2: The effect of different industrial heat treatments on the degradation of the Deltamethrin residues (ppm) in cow's milk.

Heat Treatment	No. of samples Per product	Fat% Mean ±SE	DMT (ppm) mean±SE	% Reduction
Room temp. (30 °C)	3	4.85	0.171±0.004 A	0%
63°C/ 30 min.	3	4.85	0.1451±0.009 B	15%
80°C/ 5 min.	3	4.85	0.110±0.156 C	35%
100 °C /5 min.	3	4.85	0.038±0.007 D	78%

LSD=0.0. Different capital letters in a column revealed significant (P<0.05) differences in the mean levels of Deltamethrin residues (ppm) between different heat treatments.

Deltamethrin residues in the imported UHT milk Saudi (Alsafi Dannon) and Kuwaite (KDD): Results shown in (Table, 3) revealed that all the full fat UHT milk samples had significantly (P<0.05) higher concentrations of DMT residues in summer season (0.120 ppm) than in winter season (0.025 ppm). From the obtained results, it is obvious that all of the examined full fat UHT milk samples in summer season failed to conform to the standard MRLs as they were 100% above the acceptable MRLs of 0.05 ppm recommended by (14) because most farmers used the pesticides during the summer season much more than the other seasons (15 and 16).

Deltamethrin residues in milk equipment of different capacities: Data revealed that there

was a significant (P<0.05) difference in the average mean levels of the DMT residues between milk cans of 5, 25 and 50 kg capacities and also 10 tons bulk milk tank where milk samples that were collected from milk cans (5, 25 and 50 kg) recorded significantly (P<0.05). The highest DMT residues during the summer season where their mean levels were 0.125, 0.062 and 0.046 ppm respectively in comparison to 10 tons bulk milk tank samples that had significantly (P<0.05) the lowest concentration of the DMT residues in summer season where its mean level was 0.010 ppm. (Table, 4).

Deltamethrin residues in raw milk samples of different animal species: the one way analysis of variance (ANOVA) revealed that there was a significant (P<0.05) seasonal variation of the DMT concentrations in milk samples for each animal species where all the milk samples that were collected from buffaloes, ewes, cows, goats and camels had significantly (P<0.05) higher mean levels of DMT residues in summer season (0.163, 0.133, 0.125, 0.038 and 0.032 ppm respectively) than in winter season (0.008, 0.010, 0.010, 0.003 and 0.010 ppm respectively). From obtained results, it is obvious that all of the examined milk samples from buffaloes, ewes and cows in summer season failed to conform to the standard MRLs as they exceeded the accepted MRLs of 0.05 ppm (100% violation) that recommended by the WHO and FAO for the DMT residues in milk samples as shown in (Table, 5).

From the obtained results, it was clearly obvious that the detectable concentrations of the DMT were higher in buffalo's and ewe milk samples than those found in cows, goats and camels and such results could be attributed to the higher fat content of buffalos and ewe milk than the other animals as well as the lipophilic nature of the DMT (17 and 18) reported that the pesticides residues were higher in Buffalos milk than cows milk since the fat contents of buffalo's milk was on average twice as higher as that of cows milk (14). Deltamethrin is fat soluble and either stored in adipose tissues or secreted in milk fat where residues of particularly organochlorine pesticides tend to accumulate in the body fat or enter to milk fat while the less lipophilic compounds and their metabolites may be excreted in the urine (19).

Table, 3: The seasonal variation of the mean levels of Deltamethrin residues (ppm) in imported UHT whole cow's milk samples collected from different supermarkets inside the Baghdad province.

Source of samples	No. of examined samples per season	Summer season			Winter season		
		Fat% Mean	DMT/ ppm Mean ± SE	%violation MRLs 0.05	Fat% Mean	DMT/ ppm Mean ± SE	%violation MRLs 0.05
Saudi (Alsafi Dannon)	3	4.50	0.078±0.013 Ab	100%	4.50	0.00±0.0 Bb	NV*
Kuwaite (KDD)	3	3.20	0.161±0.006 Aa	100%	3.20	0.050±0.005 Ba	NV*

LSD=0.01. Different small letters in a column revealed significant (p <0.05) differences in the mean levels of Deltamethrin (DMT) residues (ppm) between companies. Horizontal different capital letters revealed significant (p<0.05) differences in the mean levels of Deltamethrin (DMT) residues (ppm) between seasons. NV: No violation.

Table, 4: Seasonal variation of the mean levels of Deltamethrin residues in raw cow's milk samples collected from equipment of different from the milk reception and collection center in Abu-Ghraib village.

Source of milk samples	No. of examined samples per season	Summer			Winter		
		Fat%	DMT residues Mean±SE	%violation MRLs 0.05	Fat%	DMT residues Mean±SE	%violation MRLs 0.05
5 kg Can	5	3.82	0.125±0.132 Aa	100%	3. 30	0.010 ±0.003 b	NV*
25 kg Can	5	3.36	0.062±0.004 Ba	100%	3.50	0.006 ±0.0006 b	NV*
50 kg Can	5	3.25	0.046±0.008 Ca	ND*	3.60	0.005±0.0004 b	NV*
10 tons Tank	5	3.19	0.010±0.004 Da	VD*	3.00	0.0±0.0 b	NV*

LSD=0.01. Different capital letters revealed significant (P<0.05) differences in the mean levels of Deltamethrin residues (ppm) between equipment of different capacities. Horizontal different small letters revealed significant (P<0.05) differences in the mean levels of Deltamethrin residues (ppm) between seasons. NV: No violation.

Table, 5: Seasonal variation of the mean levels of Deltamethrin residues (PPM) in raw milk samples of different animal species collected from Abu-Ghraib village.

Source of milk samples	No. of examined samples	Summer			Winter		
		Fat%	DMT residues Mean±SE	% violation MRLs 0.05	Fat%	DMT residues Mean±SE	%violation MRLs 0.05
Buffaloes	5	6.85	0.163±0.003 Aa	100%	7.17	0.008 ±0.003 B	NV*
Ewes	5	6.34	0.133±0.029 Ab	100%	5.95	0.010 ±0.003 B	NV*
Cows	5	3.81	0.125±0.013 Ab	100%	3. 3	0.010 ±0.003 B	NV*
Goats	5	2.82	0.038±0.009 Ac	NV*	2.56	0.003 ±0.003 B	NV*
Camels	5	2.54	0.032±0.013 Ac	NV*	2.33	0.01±0.003 B	NV*

LSD=0.02. Means in a column not followed by the same small letters revealed significant (p <0.05) differences between animal species. Horizontal different capital letters revealed significant (p<0.05) differences in the mean levels of Deltamethrin residues (ppm) between seasons. NV: No violation.

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تحديد متبقيات المبيد الحشري دلتاميثرين في حليب الحيوانات وتاثير المعاملات الحرارية عليها

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الخلاصة

جُمعت 163 عينة (بمقدار500 مل لكل عينة) من حليب الأبقار والأغنام والماعز والجاموس والنوق جمعاً عشوائياً وعلى فترات أسبوعية وبمعدل عشر عينات أسبوعياً من مناطق أبي غريب والفضيلية مباشرة من الحيوانات ومن خزانات الحليب وحليب الثدي بالنسبة للحليب البشري كذلك الحليب المستورد المعالج بالحرارة العالية، ومن بين مجموع عينات الحليب فُحصت فقط 138 عينة حليب بجهاز كروموتوغرافي السوائل عالي الأداء في الموسمين المناخيين (الصيف والشتاء) حيث كانت المدة الأولى في الصيف والتي امتدت من بداية أيلول وحتى نهاية تشرين الأول عام 2015، فيما كانت المدة الثانية في فصل الشتاء التي امتدت من بداية كانون ثاني حتى نهاية شباط 2016. فضلاً عن تعريض بعض من العينات التي أظهرت نتائج موجبة لتواجد متبقيات دلتا مثرين إلى إحدى المعالجات الحرارية مثل 63 درجة مئوية لمدة 30 دقيقة و80 درجة مئوية لمدة 5 دقائق و100 درجة مئوية لمدة 5 دقائق لتقييم كفاءة التعرض للحرارة على تفسخ متبقيات دلتا مثرين في الحليب. وبينت النتائج ان الحليب الذي يحتوي على نسبة عالية من الدهن يحتوي على نسب مستويات أعلى لمتبقيات دلتا مثرين معنوياً ($P<0.05$) في الصيف (0.08 جزء في المليون) مما كان عليه في فصل الشتاء (0.008 جزء من المليون). أظهرت النتائج أيضاً أن جميع عينات الحليب كامل الدسم التي جُمعت في فصل الصيف فشلت لتتوافق مع الحدود القصوى القياسية لمتبقيات الدلتا مثرين والتي يمكن أن تكون خطرة لأنها تجاوزت الحدود القصوى المقبولة (0.05) جزء في المليون (انتهاك 100٪) التي أوصت بها منظمة الصحة العالمية (WHO) ومنظمة الأغذية والزراعة (FAO) بالنسبة لمتبقيات الدلتا مثرين في الحليب. عموما وبغض النظر عن أنواع الحيوانات والموسم فقد أشارت النتائج إلى أن عينات الحليب التي احتوت على نسب عالية من الدهون أظهرت بقوة وجود تراكيز عالية من متبقيات الدلتا مثرين وبعبارة أخرى ان زيادة نسبة الدهون في الحليب لها علاقة طردية مع مستويات متبقيات الدلتا مثرين نظرا لكون مبيد الدلتا مثرين محب للاذابة بالدهون. وكشفت نتائج التحليل الإحصائي بان عينات الحليب التي تم جمعها من الجاموس، النعاج والأبقار سجلت فرقاً معنوياً ($P<0.05$) وارتفاعاً لمتبقيات الدلتا مثرين لكلا الموسمين حيث كان متوسط مستوياتها التي تجاوزت الحدود القصوى المقبولة من (0.05) جزء في المليون مقارنة لعينات حليب كل من الماعز والإبل والتي سجلت فرقاً معنوياً ($P<0.05$) وانخفاضاً في المستويات المتوسطة من متبقيات الدلتامثرين. ان عينات الحليب التي جمعت من حاويات الحليب ذات سعة (5 و25 و50 كيلو غرام) سجلت فرقاً معنوياً ($P<0.05$) كان أعلى نسبة لمتبقيات الدلتا مثرين في فصل الصيف في حين خزانات الحليب ذوات سعة 10 طن قد سجلت فرقاً معنوياً ($P<0.05$) وانخفاضا في تركيز متبقيات الدلتامثرين في فصل الصيف.

الكلمات المفتاحية: كروموتوغرافي السوائل عالي الأداء، الدلتامثرين، الحليب كامل الدسم، الحليب المنزوع الدسم.