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The impact of seasonal variation on organochlorine pesticide residues in buffalo and cow milk of selected dairy farms from Faisalabad region

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Abstract Two hundred milk samples from 20 randomly selected dairy farms were screened for the incidence of organochlorine pesticide residues to evaluate the safety of milk in Faisalabad region. The results revealed that overall buffalo milk samples in winter (85 %) and in summer (78 %) were more contaminated as compared to cow milk samples 83 and 75 % in respective seasons. The residues of cyhalothrin were found only in summer season in milk of both species. Permethrin residues were detected at higher levels than perfinofos while DDT and methamedophos were found undetectable. The mean levels of permethrin were 0.042 and 0.033 mg kg^{-1}in buffalo milk samples and 0.045 and 0.043 mg kg^{-1} in cow milk in winter and summer season, respectively. Perfinofos residues were found to be the least contaminated pesticides with mean values of 0.0006 and 0.0013 mg kg^{-1}, respectively in winter season, and 0.004 and 0.0025 mg kg^{-1} in summer season. All analysed pesticide residues in milk samples in both seasons were below the maximum residual limit (MRL) values as described by European Union (EU) but milk samples contaminated with α, β-endosulfan and endosulphate exceeded their respective Food and Agriculture Organization's (FAO) established MRLs both in winter and summer.

Keywords Pesticide residues · Dairy farms · Milk · Seasons · GC-ECD

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Introduction

In the twentieth century of intensive agricultural and farming, the use of pesticides has emerged as a viable option in reducing the drastic impacts of food shortage and consequent problems. Besides the fact that pesticides could upset the environment, some countries allow their use to enhance the productivity of small holder farming (Ciscato et al. 2002; Deti et al. 2014) and developing countries cannot afford to ban these certain chemicals for reasons of cost and/or efficacy. In consequence, these synthetic chemicals have been remained in practice at large quantities (Gebremichael et al. 2013). Among pesticides, organochlorine pesticides (OCPs) are a family of man-made persistent chlorinated compounds to which humans are most likely exposed. These compounds are lipophilic in nature and possess the property of bioaccumulation. They may enter the human body via food stuff of animal origin which majorly contributes the OCPs intake (about 95 % of human intake) (Darnerud et al. 2006; Focant et al. 2003). OCPs resist to degradation and have long stability that results in damaging the environment and human health (Chao et al. 2006). Species at higher trophic level in food chain are more prone to contract of contamination by these tenacious chemical compounds. Once absorbed from intestine to systematic circulation, they tend to concentrate in high fat tissues and milk (Tsiplakou et al. 2010; LeDoux 2011). Subsequently, diet paves the pathway for chronic exposure to these toxic chemicals to humans. Milk is considered as pivotal part of daily diet, and it is regularly consumed by people of all age groups. At the same time, milk also offers an ideal capability of dissolving the environmental contaminants such as pesticides (owing to their fat-soluble nature). Although there is public concern on the use of pesticides, especially OCPs, yet studies on residual levels and their effects are limited (Kampire et al. 2011). Contamination of milk with OCPs residues is very much alarming and needs timely judicious as these pollutants are endocrine disrupting chemicals. Furthermore, they are thought to pose a wide range of health effects such as infertility and fecundity, reproductive abnormalities, neurobehavioral dysfunctions, skewed sex ratios and impaired immune function. Kids and young people relying on dairy milk are more vulnerable to adverse health effects of these chemicals (McKinlay et al. 2008; Windham et al. 2005).

Pakistan is occupying the fourth position (49.512 million tonns per annum) among milk-producing countries in the world ranging behind India, China and USA (Asi et al. 2012; Iqbal et al. 2011). OCPs are still used in Pakistan and their presence in different food commodities have been reported in several parts of the country (Parveen et al. 2005; Sanpera et al. 2002; M. Y. Tariq et al. 2006; M. I. Tariq et al. 2007). However, limited data is available on the presence of these pesticides residues in milk. Keeping in view this rationale, and to circumvent the subsequent health impacts of OCPs residues, it is of utmost importance that the levels of OCPs should be monitored in milk. Therefore, the present study aimed to investigate the levels of OCPs in milk collected from different farms during summer and winter seasons.

Material and methods

Standards and reagents

Analytical technical grade pesticide standards were supplied by Dr. Ehrenstorfer GmbH, Augsburg, Germany. Florisil solid-phase extraction columns were purchased from Agilent Technologies, Inc. (USA). Methanol, petroleum ether, anhydrous sodium sulphate and other chemicals used in this study were of analytical grade and purchased from Sigma Aldrich (St. Louis, MO, USA).

Milk sample collection

Fresh milk samples were collected from 20 different farms (10 buffalo and 10 cow dairy farms) in the vicinity of Faisalabad within a radius of 45–50 km with frequency of five samples per farm during each season. A total of 50 samples for buffalo and 50 samples for cow were collected in winter, and in a similar way 50 samples each of buffalo and cow were collected in summer. Briefly, during summer and winter season, samples were collected from March to July 2013 and from October to February 2014, respectively. Milk samples were collected in sterilized glass bottles and transported to the lab in ice box, where milk was analysed immediately or stored at −20 °C for further analysis.

Extraction and clean up

Extraction of pesticide residues from milk samples was carried out as described elsewhere previously (Kampire et al. 2011) with slight modifications. Briefly, after homogenization, milk sample (50 mL) was taken and centrifuged at 5000 rpm for 20 min at 4 °C. The upper fat layer was removed, weighed and 1 g of fat was taken in separate clean flask containing 30 g of anhydrous sodium sulphate. Two hundred milligrams of petroleum ether was added and the mixture was shaken gently. Subsequently, the mixture was filtered and the extract was evaporated to 1 mL by rotary evaporator. The cleanup was performed using glass column, pre-rinsed with 7 mL hexane, packed with florisil (10 g) and topped with anhydrous sodium sulphate (5 g). The extract was (not the column) eluted using 150 mL of n-hexane. After elution, the extract was evaporated to ~1 mL and flushed with a gentle stream of nitrogen at 40 °C to dryness. The residue was dissolved into 1 mL methanol and filtered through 0.2 μm filter paper, and final volume of 1.5 mL of the sample was analysed by gas chromatography (GC).

Gas chromatography analysis

The pesticide residues were analysed using a gas chromatography (Agilent Model 6890) equipped with a ^{63}Ni electron capture detector. A HP-5MS capillary column (30-m length; 0.25 mm i.d; 0.25-μm film thickness) was used. Two microliter of extract was injected in splitless mode into a fused silica capillary column using N_2, a carrier gas, with a 1.0 mL/min flow rate. The GC oven temperatures were programmed as: initial temperature of 150 °C held for 5 min, then ramped to 210 °C at a rate of 8 °C min^{-1} and maintained at this temperature for 2 min. Finally, the temperature was increased to 300 °C at a rate of 15 °C min^{-1} with the hold time of 10 min. The detector and injector temperatures were 300 and 280 °C, respectively.

Method validation

Standards of analysed pesticides were run and standard curves were drawn by using internal and external standardization method for quantification of pesticide residues. Bifenthrin was used as internal standard that was added in samples at concentration of 0.1 ppm. The method validation was calculated by recovery studies of all pesticide residues in this study. The limit of detection (LOD) was 0.0001 to 0.0012 mg kg^{-1} and the limit of quantification was 0.0003 to 0.0038 mg kg^{-1} (Table 1). The LOD calculated by determining the concentration of pesticide residues that is three times to the signal produced and 10 times to the signal produced by noise using root-mean-square-error (RMSE) method (Johannes, 2003). Recoveries were carried out by spiking blank samples with each pesticide in the study at the rate of 0.5 and 1.0 mg kg^{-1}. Blank samples were determined by GC/ECD without any pesticides and/or having residues lower than LOD. Recoveries were found to be within the range of 80–110 %.

Statistical analysis

The statistical analysis was performed using Microsoft excel 2010 software. The concentration of pesticides residues in milk samples expressed in milligram per killogram as mean plus standard deviation (SD). Analysis of variance (ANOVA) was carried out between farms, seasons and pesticide residues with Tukey pairwise comparison using Minitab Table 3.

Table 1 Limit of detection and limit of quantification for the selected pesticides analysis

Pesticides	LOD (ppm)	LOQ (ppm)
Cyhalothrin	0.0012	0.0038
Deltamethrin	0.0002	0.0008
α-Endosulfan	0.0008	0.0020
β-Endosulfan	0.0006	0.0019
Endosulphate	0.0004	0.0015
Bifenthrin	0.0002	0.0018
Permethrin	0.0003	0.0010
Cypermethrin	0.0005	0.0014
Chlorpyrifos	0.0001	0.0005
DDT	0.0001	0.0004
Perfinofos	0.0001	0.0003
Methamedophos	0.001	0.0031

LOD limit of detection, *LOQ* limit of quantification, *PPM* part per million

Results and discussion

In the present study, milk samples from 20 different dairy farms of two milch species (buffalo and cow) from Faisalabad region; Punjab were analysed to determine the levels of OCP contamination in summer and winter seasons. The dairy farms were selected randomly from all sides of Faisalabad district (covering as much as possible area) for the safety assessment of milk for consumer consumption. The results of milk samples were summarized in Table 2. Cyhalothrin residues were only detected in the summer season in both dairy farms with the mean values 0.021 and 0.011 mg kg^{-1}, respectively. In both seasons, endosulphate was not detected in cow milk whereas in buffalo milk its contamination level was found higher than FAO established MRL value. The DDT and methamedophos residues were not detected in any of the milk samples for both species during the two seasons. The collected milk samples were found contaminated with endosulfan isomers of α, β, deltamethrin, bifenthrin, permethrin, cypermethrin, chlorpyriphos and perfinofos in both seasons. Eighty-five percent of the buffalo milk samples were found to be contaminated in winter season and 78 % in summer whereas 83 % of cow milk samples were detected as contaminated with pesticides in winter season and 75 %

Table 2 Pesticide residue in Bufallo and cow milk

Pesticides residues	Buffalo milk		Cow milk		MRLs	
	Winter Mean ± SD (mg kg^{-1})	Summer Mean ± SD (mg Kg^{-1})	Winter Mean ± SD (mg kg^{-1})	Summer Mean ± SD (mg kg^{-1})	EU	FAO
Cyhalothrin	ND	0.021 ± 0.0183	ND	0.011 ± 0.009	0.05	0.2
Deltamethrin	0.022 ± 0.0029	0.0115 ± 0.007	0.018 ± 0.003	0.0091 ± 0.0003	0.05	0.05
α Endosulfan	0.034 ± 0.0015	0.0238 ± 0.007	0.036 ± 0.003	0.026 ± 0.007	0.05	0.01
β Endosulfan	0.036 ± 0.001	0.032 ± 0.007	0.031 ± 0.019	0.018 ± 0.003	0.05	0.01
Endosulphate	0.039 ± 0.004	0.025 ± 0.005	ND	ND	0.05	0.01
Bifenthrin	0.025 ± 0.003	0.011 ± 0.004	0.019 ± 0.011	0.024 ± 0.006	0.2	0.2
Permethrin	0.042 ± 0.016	0.033 ± 0.006	0.045 ± 0.015	0.043 ± 0.013	0.05	NE
Cypermethrin	0.034 ± 0.015	0.021 ± 0.006	0.0299 ± 0.015	0.027 ± 0.008	0.05	0.05
Chlorpyrifos	0.005 ± 0.0007	0.004 ± 0.0009	0.004 ± 0.0005	0.003 ± 0.0007	0.01	0.02
DDT	ND	ND	ND	ND	0.02	0.02
Perfinofos	0.0006 ± 0.0001	0.0004 ± 0.001	0.0013 ± 0.0017	0.0025 ± 0.0005	0.01	0.01
Methamedophos	ND	ND	ND	ND	0.01	0.02

SD standard deviation, *ND* not detected, *MRL* maximum residual limit, *EU* European Union established MRL, *FAO* Food and Agriculture Organization established MRL, *NE* not established

in summer, respectively. All of the analysed pesticides in milk samples in both seasons were below the EU described MRL values, but milk samples contaminated with α-, β-endosulfan endosulphate exceeded their respective FAO MRL limits. Out of 50 buffalo samples, 27 in summer and 34 in winter exceeded their MRL for α-, β-endosulfan, endosulphate; 20 milk samples in summer and 24 in winter exceeded their MRLs for α-, β-endosulfan established by FAO. Permethrin was detected at higher levels with the mean of 0.042 and 0.033 mg kg^{-1} in buffalo milk samples in winter and summer, respectively; and 0.045 and 0.043 mg kg^{-1} in cow milk samples in winter and summer season.

Perfinofos residues were found to be the least contaminated pesticides with mean values of 0.0006 and 0.0013 mg kg^{-1}, respectively in winter; and 0.004 and 0.0025 mg kg^{-1} in summer (Table 2). ANOVA showed that interaction of season and pesticides; farms and pesticide residues; seasons, farms and pesticide residues have significant difference Table 3. Tukey pairwise comparison showed that chlorpyrifos was found maximum in winter season from buffalo farm while permethrin was found maximum in winter season form cow farm. The detected pesticide residues were higher in buffalo milk compared to cow milk in winter except for α-endosulfan and permethrin. In summer, α-endosulfan, bifenthrin, permethrin, cypermethrin and perfinofos were detected higher in cow milk in comparison to buffalo milk (Fig. 1). Several studies have been conducted on pesticide residues throughout the country reporting different pesticide residues in feed, cottonseed, fruit, vegetable and fish and other similar food commodities (Ali et al. 2015; Asi et al. 2012; Kumar et al. 2014; Mumtaz et al. 2015; Robinson et al. 2016; Sultana et al. 2014; Zehra et al. 2015). Milk from both species of milch animals was found contaminated with pesticide residues, and the possible ways of exposure of animals to these residues include fodder, contaminated soil, ground water, air, and neighbour farm. (Saqib et al. 2005; M. Y. Tariq et al. 2006). Permethrin residues were detected at higher levels, and it may be due to the fact of its higher use as foliar spray on fodder. Cyhalothrin was found only in summer season due to the practice of foliar application of

Table 3 Relation of pesticide residues with seasons and farms

SOV	α-value	DF	*P* value
Seasons	0.05	1	0.088
Farms	0.05	1	0.00
Pesticide residues	0.05	9	0.00
Seasons × farms	0.05	1	0.693
Seasons × pesticide residues	0.05	9	0.000
Farms × pesticide residues	0.05	9	0.000
Seasons × farms × pesticide residues	0.05	9	0.000

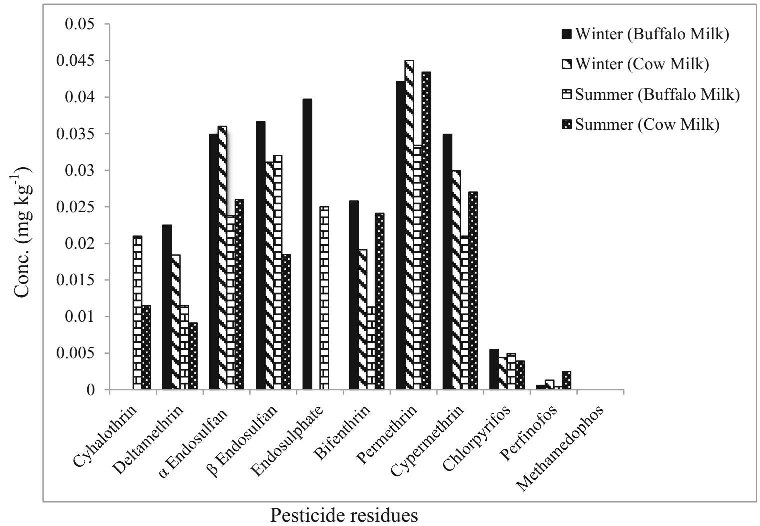

Fig. 1 Comparison of pesticide residues in buffalo and cow's milk in winter and summer seasons

cyhalothrin on fodder to protect against insect pests especially in summer season. Endosulphate was only found in buffalo milk because it tends to have more solubility in fat (buffalo milk has more fat contents) as compared to cow milk which has low fat. There were no traces of DDT and methamedophos residues detected in milk. The reason is that there is an imposed ban on DDT usage for agricultural crops and methamedophos that belong to organophosphate group pesticide which has low persistent in the environment.

In conclusion, buffalo milk samples were more contaminated compared to cow milk samples. Furthermore, the residues were found to be higher in winter than in summer season. It may be the reason that summer season has characteristic of high temperature, rain, winds which may help in reducing the residual effect of pesticides in milk. Pesticide residues can be leached down by rain water; high temperature can decompose the pesticide residues to some extent resulting less pesticide residues in summer. DDT and methamedophos were found undetectable, whereas the permethrin and perfinofos were the highest and lowest contaminated residues, respectively.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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