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Quantification of pesticide residues in cow milk samples collected from district Sargodha Punjab, Pakistan

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ABSTRACT

Milk and milk products are known to have high nutritional value. Milk is a source of energy and it contains all required building proteins, bone forming minerals and fats. Milk is contaminated with pesticides and consumption of contaminated milk, meat and dairy products is the cause of high level of pesticides in the human body. Milk is a good source of dissolving pesticide residues which are fat soluble. Present study was designed keeping in view the nutritional importance of milk and its contamination through pesticides application. For this purpose, raw cow milk samples from citrus plantation zone in district Sargodha were collected and analyzed for the presence of deltamethrin and malathion pesticides using HPLC technique. Results showed that 100 % of milk samples were contaminated with pesticide residues. Maximum concentration of malathion (0.72±0.02 µg/kg) and deltamethrin (0.75±0.04 µg/kg) was found in cow milk samples collected from Sargodha. Minimum pesticide residues were found in cow milk collected from Tehsil Bhera. Both deltamethrin and malathion concentration in milk samples varied in all zones. None of the samples showed concentration higher than MRL prescribed by FAO. These results are of concern as adverse health effects exerted by deltamethrin and malathion are well known. The results of this study confirm the risks of pesticide residues exposure that threaten consumer health in all districts of Sargodha. So, it is recommended that the pesticide residue monitoring programs must be instituted in Pakistan.
Keywords: Cow milk, HPLC, Multi pesticide residues, Pakistan

INTRODUCTION

Increase in world population has led to agricultural escalation (Merrington 2004). Agricultural chemicals, mainly pesticides, are used to increase the production to meet food requirements. If pesticides are not applied, crop yield reduces to 37-79% (Behra & Singh 1999). Therefore use of pesticides is inevitable. On a global scale, Europe and USA consume 69% of the total pesticides produced each year (Aktar 2009). Besides agricultural uses, pesticides are used to control the diseases such as malaria in developing countries (Deti 2014).

South Asia houses 14% of the world's productive land; pesticides are extensively used in this region to gain economic benefits through increasing crop production (Atapattu & Kodituwakku 2009). Pesticides have been in use in Pakistan since 1965. In Pakistan use of pesticides has increased to 11.69 % in last 20 years (Tariq *et al* 2007). Pakistan imports thousand tons of pesticides from Europe and USA (Ahad 2010) for the malarial eradication, locust control and pest invasion (Ali 2013). Pakistan banned the use of pesticides but these are still in circulation. The use of banned pesticides is due to poor legislation (Jan 2009).

Eighty percent of pesticides are sprayed only on cotton plants (Economic survey of Pakistan, 2005-2006), therefore these areas are heavily loaded with pesticide residues (Tariq 2005). Most pesticides are volatile due to which they have the ability to travel in air. After condensing and aggregating in air, these residues fall on the earth surface in the form of rain. By this way they contaminate water and soil. Due to their transportation capability

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(Harner 2001) pesticide residues reach those areas where they have never been used (Zhang 2008). The Organo chlorine pesticides (OCPs) were very effective pesticides or insecticides used worldwide until restrictions were announced by developed countries (Ashangar 2009). These are generally characterized by low volatile and lipophilic nature (Bedi 2015). OCPs are noxious environmental contaminant as they are highly stable and persistent. They cause bioaccumulation especially in high fat containing foods such as milk and dairy products (Tsakiris 2015).

Organo phosphorous pesticides (OPPs) are widely used as insecticides. OPPs are the esters, amides, or thiol derivatives of phosohoric acid. These pollutants have shorter persistence in environment as they hydrolyze easily (Shaker & Elsharkawy 2015). Yet OP pesticides pose a serious health risk, especially fat-soluble OPs. Their residue accumulates in animal fat tissue, and milk (Fagnani 2011). Chronic residues of pesticides cause environmental contamination. Due to their slow degradation process these residues are reported in air, water bodies and soil ultimately getting entrance in the food chain (Khwaja 2007).

The existence of pesticide residues in environment is a burning issue in developing countries like Pakistan. Their presence in environment is due to sluggish financial development (Hassan 2014) and lack of synchronization among governing bodies (Bhambhro 2004). Presence of these pesticide residues in food is hazardous to public health and wildlife. Pesticides affect the nervous system of insects, calcium metabolism in birds (Ashnagar 2009). Discontinuous exposure to perilous pesticides causes acute illness such as headache, nausea and abdominal pain. Continuous exposure to pesticide produces chronic effect such as cancer and reproductive failure (UNEP 2003). Pesticide residues are the causative factor for many diseases such as Parkinsonism (Muhammad 2012) and Alzheimer (Aslam 2013). These are reported as mutagenic and teratogenic to human (Castilla 2010). They also have been reported as anti-androgen in males and carcinogenic in females (Patricia & Paul 2004) causing sterility and breast cancer respectively. They have an effect on the lipid metabolism and transport of vitamins and glucose in human (Avancini 2013).

High values of pesticides were detected in fruits and vegetables (Mebdoua 2018). About 90% of pesticide contamination in human diet is due to the daily intake of animal based products (Tecle 2013). Products of animal origin such as milk, cheese and butter (Saleem 2009) can easily be contaminated with pesticides due to their high lipid content (Kampire 2011). Primarily pesticides are stored in fat rich tissue and afterward translocated and excreted in the form of milk fat. Milk is an essential and complete food, containing protein, fat and major minerals (Hassan 2014). Milk and milk product are the key source of calcium, riboflavin (vitamin B2), phosphorus and iodine in the diet.

Generally, milk has been studied as the indicator of bioconcenteration of pesticides in environment. Pesticides accumulate in milk producing animal by means of contaminated feed and air. Due to their lipophilic property, these are mainly accumulated in fat tissue. Pesticide residues subsequently translocated and excreted in milk through milk fat (Waliszewski 1997). Milk contains about 20% of ingested pesticide residues (Khaniki 2007). In order to promote trade and protect consumers from the deleterious effect of pesticides it is necessary to find out their concentration in edible products i.e. milk. Therefore MRL (maximum residue level) of pesticide residues for milk and other products have been established by developed countries (Muhammad 2012). If residues are above the MRL then this pose a serious health concern to humans and wildlife (Sanpera 2003). Existence of pesticide residues in milk is a matter of great concern all over the world. The objective of present study was to determine the concentration of pesticide residues in cow milk samples collected from different localities of Sargodha, Punjab, Pakistan. This study will be helpful for both common people and farmers that they should use pesticides with great care.

MATERIALS AND METHOD

Sample collection

One hundred and eighty raw cow milk samples were collected from all tehsils (six) of district Sargodha (32.0740° N, 72.6861° E), Punjab, Pakistan. Milk samples were collected in clean and sterilized plastic bottles. Sampling was carried out during the period of November 2018 to January 2019. Milk samples were kept immediately in ice after collection, transferred to laboratory and preserved at -20°C until analysis.

Chemicals and reagents

All chemicals used in the study were of analytical grades. Sodium acetate, acetic acid (99.5%) and methanol were purchased from Sigma Aldrich.

Sample preparation

Sample preparation and analysis method administrated in present study were those reported by Cardeal & Clauda (2006) with slight modifications. Buffer solution (pH 4.5) was prepared by mixing 6.80 g of sodium acetate and 2.87 ml of acetic acid (99.5%) and added distilled water till 1000 ml. 20 ml milk sample was taken and added 27 ml buffer solution. Sample was centrifuged at 15000 rpm for 15-20 minutes. Protein was precipitated and 16 ml aliquot of upper layer was transferred to 20 ml of glass vial. Supernatant was used for further analysis.

Clean up setup

Solid phase micro extraction (SPME) with dimethyl siloxane (PDMS) fiber (100 µm thickness) was purchased from Supelco (Bellefonte, PA, USA). The fiber was immersed into the sample. Fiber was stirred continuously in sample for half an hour then removed and immersed into methanol (70 %) for elution of pesticides residues.

HPLC analysis

High performance liquid chromatography (HPLC) system (Shimzadu FLC LC-20AD model) equipped with UV-Vis detector used for the analysis of pesticides. The 20 µl of eluted methanol was introduced into HPLC with the help of injection. Thermohypersil-C18 column was used for analysis of residues. A wavelength of 235 nm was used to get absorption spectra. Mobile phase used in HPLC was the mixture of acetonitrile and methanol (11:9 v/v %).

Statistical analysis

Microsoft Excel 2007 and SPSS software (V.18) were used to perform statistical analysis. Analysis of variance and Tuckey test were used to evaluate the concentration differences between pesticide residues. Differences were considered significant at values of $p < 0.05$.

RESULTS AND DISCUSSION

A total 180 cow milk samples were collected from six Tehsils (three farms from each Tehsil) of District Sargodha, Punjab. The samples were analyzed for malathion and deltamethrin residues. Residue levels of deltamethrin and malathion in each sample were calculated (in µg/kg Table 1, 2). Statistical analysis showed that deltamethrin (0.75±0.04 µg/kg) was most notably present in milk samples collected from Sargodha. The mean concentration of deltamethrin in milk sample collected from Bhalwal was less than that of Sargodha whereas its value was greater than Bhera, Silanwali, Kotmomin and Shahpur. Maximum value of Malathion was found in cow milk (0.72±0.02 µg/kg) collected from Sargodha. Minimum Malathione residues found in cow milk (0.32±0.02 µg/kg) collected from Tehsil Bhera. Both deltamethrin and malathion concentration in milk samples varied in all zones.

Results revealed that 100% of analyzed cow milk samples were contaminated with deltamethrin and malathion indicating their presence and risk in the environment. Deltamethrin and malathion levels were higher in milk samples collected from the Sargodha than from all other regions. It might be due to the fact that Sargodha is well known for citrus fruits and pesticides are heavily sprayed on citrus plants. Eventually these pesticide residues enter the environment. Furthermore, levels of deltamethrin (0.41-0.78 µg/kg) and malathion (0.29-0.76 µg/kg) in milk samples were below the MRLs prescribed by FAO/WHO Codex Alimentarius 2008. It might be due to short half-life of pyrethroids as these easily degrade in milk (Ahmad 2012). But the presence of deltamethrin in milk may prove hazardous to human health. Therefore, it is recommended to conduct a study to check the prevalence of pyrethroids and OPPs in these agriculture dominant areas. Lowest mean concentration of deltamethrin (0.43±0.01 µg/kg) and malathion (0.32±0.02 µg/kg) was found in milk samples collected from the Kotmomin and Bhera, respectively. There are fewer citrus orchards in Bhera, this might be the reason for low level of pesticides in milk collected from there.

The levels of pesticides in this study were higher than those detected by Bedi (2015) which was 0.5±3.4 µg/kg and 0.4±3.9 µg/kg in milk for deltamethrin and malathion, respectively

and then those reported by Hassan (2015). According to study 7% of samples were positive in concentration less than the MRL. It might be due to the continuous use of pesticides at the locations from where the samples were collected.

In current study, residue levels of deltamethrin were less than the levels obtained by Shahzadi *et al* (2015) where 50% of the milk samples were contaminated by different pesticides; deltamethrin being present at highest concentrations (0.57 µg/kg) amongst all studied pesticides who studied the residue levels of different pesticides in milk (goat, cow, buffalo, sheep and camel).

Parveen (2006) reported that 63% and 40% of milk samples were contaminated with pesticide residues, respectively. These residues might have been derived from the fodder or from pesticides used to control ecto parasites on cattle. Residual level of malathion detected in milk samples was 0.09±0.01 mg/kg in Egypt. However, its value was higher than our results (Shankar 2015). Furthermore, residue levels measured in our study were lower than those reported by Fagnani (2011) who found 0.02 mg/kg of malathion in milk samples. Muhammad (2010) found that 20-25% milk samples super passed the MRLs levels for OPP and pyrethroids. While the levels of malathion in the samples analyzed in this study were higher than the MRL prescribed by EC (European Commission). In Mexico, Salas (2003) reported that 39.5% of pasteurized milk samples were contaminated by OPP residues. Yet, Malathion residues in milk samples were less than that in our results. However, in present study malathion was detected in cow milk samples. The reason could be the continuous use of pesticides in the regions from where milk samples were collected.

For the first time a study was conducted in Tehsils of district Sargodha to investigate the presence of pesticide residues in cow milk. Present study provided preliminary information on concentrations of Deltamethrin and Malathion in cow milk. Results indicated the recent use of pesticides on crops, cattle and feed stuff. Therefore, it is recommended to conduct a research to determine the residues of pesticide in fodder and feed of lactating animals.

Table 1: Mean (S.E.) values of malathion (µg/kg) in cow milk collected from different localities, Sargodha, Punjab

Localities	Farm 1	Farm 2	Farm 3	Mean±S.E	Range
Bhalwal	0.68 ±0.02	0.64±0.03	0.60±0.01	0.64±0.03	0.60-0.70
Silanwali	0.38±0.03	0.36±0.01	0.36±0.03	0.37±0.02	0.33-0.42
Bhera	0.32±0.03	0.34±0.02	0.31±0.03	0.32±0.02	0.29-0.36
Sargodha	0.70±0.02	0.73±0.02	0.74±0.01	0.72±0.02	0.68-0.76
Kotmomin	0.35±0.03	0.36±0.03	0.33±0.02	0.34±0.02	0.31-0.39
Shahpur	0.42±0.02	0.45±0.03	0.43±0.02	0.43±0.02	0.40-0.48

Table 2: Mean (S.E.) values of deltamethrin (µg/kg) in cow milk collected from different localities, Sargodha, Punjab

Localities	Farm 1	Farm 2	Farm 3	Mean±S.E	Range
Bhalwal	0.62±0.02	0.54±0.02	0.58±0.02	0.58±0.03	0.52-0.64
Silanwali	0.56±0.03	0.51±0.02	0.47±0.03	0.52±0.04	0.44-0.59
Bhera	0.5±0.02	0.45±0.01	0.52±0.03	0.49±0.03	0.44-0.55
Sargodha	0.75±0.04	0.78±0.03	0.72±0.01	0.75±0.04	0.68-0.78
Kotmomin	0.42±0.02	0.43±0.02	0.43±0.01	0.43±0.01	0.41-0.45
Shahpur	0.56±0.02	0.53±0.01	0.51±0.02	0.54±0.02	0.51-0.58

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