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PESTICIDE RESIDUES IN COW MILK AND DAIRY PRODUCTS FROM THE MAJOR MILK PRODUCING AREA OF SRI LANKA

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ABSTRACT

Nuwara Eliya district is the leading fresh milk producing area in Sri Lanka. In the district, pesticides are widely applied for intensive cultivation of vegetables which leads to contamination of water and material used to feed cows. Contamination and health risk hazards of organophosphorus pesticide residues in milk and dairy products originated in the district were studied. Identification and quantification of eleven commonly used pesticides in 50 milk samples and 12 dairy product samples were performed using standard analytical methods and GC-MS technique. Results revealed that fresh milk contained residues of Prothiofos (0.0568±0.037 mg/kg), Diazinon (0.0378±0.009 mg/kg), Chlorpyrifos (0.0264±0.004 mg/kg), Profenofos (0.196±0.099 mg/kg), Fipronil (0.1906±0.188 mg/kg), Phenthoate (0.1012±0.110 mgkg), Dimethoate (0.1196±0.201 mg/kg) and Tebuconazole (0.062±0.069 mg/kg) at higher levels than the recommended maximum residue levels (MRLs) of the World Health Organization. Sterilized milk (0.0115±0.000 mg/kg) and fermented milk (0.022±0.004 mg/kg) contained higher levels of Profenofos than the MRLs. Higher levels of Fipronil than MRLs were observed in pasteurized milk (0.086±0 mg/kg) and fermented milk (0.014±0.000 mg/kg) samples. Phenthoate at higher levels than MRL was reported in pasteurized milk (0.3645±0.402 mg/kg), sterilized milk (0.1405±0.197 mg/kg) and milk powder (0.0055±0.000 mg/kg). Moreover, Dimethoate content in fermented milk (0.087±0.012 mg/kg) was higher than the MRL. Routine monitoring of the above pollutants in food items including fresh milk and value added milk products is essential to prevent, control and reduce the pollution and to minimize the health risks to consumers.

Key words: *Pesticide residues, Cow milk, Dairy products, Health risk*

INTRODUCTION

Organophosphorus pesticides have been extensively used in Sri Lanka to increase crop production. Especially, farmers in the central part of the island who cultivate upcountry (elevation >900 m of sea level) vegetables continuously using intensive cultural practices with hybrid varieties used to apply pesticides beyond the manufacturers recommended levels (Dilhani *et al.*, 2015). Intensive usage of pesticides has resulted in trace contamination of air, water and soil with their residues (Pandit *et al.*, 2002). The residues of these pesticides can be absorbed by milk producing animals such as cows through contaminated feed, water and inhaled air (Pandit *et al.*, 2002; Ravichandran *et al.*, 2015). Milk is the most versatile organic food product of animal origin (Ghidini *et al.*, 2005). Pesticide residues being highly lipophilic are primarily stored in fatty tissues in cows and later excreted through milk fat. As a result, consumers are at a risk of exposing to these pesticide residues as they are accumulated in fresh milk and fat rich dairy products (Nigam and Siddiqui, 2001; Ravichandran *et al.*, 2015). Most organophosphorus pesticides undergo degradation by hydrolysis, yielding nontoxic, water soluble products. Therefore, toxic hazards of organophosphorus pesticides are short term, however, they show higher acute toxicities (Darko and Akoto, 2008). The inhibition of acetylcholinesterase in the nervous system, resulting in respiratory, myocardial and neuromuscular transmission impairment is the toxicological effect of the organophosphorus pesticides (Goh *et al.*, 1990). Therefore, pesticide residues in food represent a significant health risk (Darko and Akoto, 2008).

Nuwara Eliya district, which is located in the central area of the island, is producing the highest amount of fresh milk (72 Mn l/yr) in Sri Lanka (Department of Animal Production and Health, 2015). A large number of milk farmers sell fresh milk to processors in the district for manufacturing dairy products. It is a common practice that milk farmers in the Nuwara Eliya district use crop residues and grass from the surrounding area to feed their cows (Dilhani *et al.,* 2015). Moreover, they use surface and ground water sources to feed cows which could be easily contaminated with the intensive application of pesticides for vegetables (Pathirana *et al.*, 2015). The effect of regular intake of pesticide residues in food is hard to detect and quantify. However, management and regulation of these chemicals are vital considering the quality of milk and the risks associated with human health. This study therefore, seeks to provide the baseline information on the contamination levels of organophosphorus pesticide residues in fresh milk and dairy products originated in the main fresh milk producing area of Sri Lanka.

MATERIALS AND METHODS

Chemicals and reagents

Pure and mixture of standards were purchased from Dr. Ehrenstofer Co. (Augsburg, Germany). These certified pesticide standards contained greater than 98% purity. Internal standard, Triphenyl phosphate was purchased from

AccuStandard, USA. Florisil (60-100 mesh) was purchased from Fluka Analytical. All other solvents and reagents were purchased from Sigma Aldrich.

Milk and dairy products sampling

The list of milk collecting centers belonged to the government and private sector located in the Nuwara Eliya district was obtained from the District Veterinary Office, Nuwara Eliya. Fresh milk samples were taken from milk chilling tanks of 62 randomly selected milk collecting centers. Samples were collected in to clean and sterile amber colored plastic bottles. The milk samples were kept on ice immediately after collection and transferred to the Laboratory and stored at -4^0C till the time of analysis. Four types of dairy products namely pasteurized milk (UHT milk), sterilized milk (Bottled milk), fermented milk (Yoghurt) and spray dried milk (Full cream milk powder) originated from Nuwara Eliya district were collected from randomly selected retail outlets located in the district. These samples were stored at 4-6^0C before subject to further analysis.

Preparation of standard solutions

Stock standard solutions of pesticides were prepared at a concentration of 500 mg/l in acetone and methanol, depending on the compound's solubility. Stock solution of internal standard, Triphenyl phosphate was prepared at a concentration of 500 mg/l. A working standard solution of pesticides and internal standard at the concentrations of 5 mg/l were prepared. The stock and working standard solutions were stored at 4^0C until needed.

Quality control and quality assurance

To determine the method quality the linearity, recoveries, limits of detection (LOD) and limit of quantification (LOQ) were tested. The limits of detection and of quantification of each substance were determined initially with standard solutions. LOD was calculated as three times the signal-to-noise ratio. LOQ was calculated as 10 times the signal-to-noise ratio.

Extraction and cleanup of milk samples

A chromatographic tube was filled with 100 ml petroleum ether and 25 g of standardized Florisil was slowly added. The adsorbent was allowed to settle, and petroleum ether was drained to a level of about 50 ml above the top of the adsorbent. A total of 25 g Florisil was added in small portions to the milk sample (10 g). While adding the Florisil, milk sample was stirred continuously with a glass rod until a homogenous, free-flowing powder was obtained. Then resulted powder was packed to the column, washed with petroleum ether and the same was collected in a 1-1 round-bottomed flask. Then the column was eluted with 300 ml of the eluting mixture at a flow rate not exceeding 5 ml/min, and collected elute in the same flask. Elute was rotary-evaporated and concentrated to about 5 ml. Last traces of solvent were removed with the aid of a gentle stream of air. The remaining residue after evaporation was transferred with a small volume of

petroleum ether into a 5 ml volumetric flask and diluted to the mark. Thereafter, the sample was transferred to a gas chromatography vial and stored in refrigerated condition until use for analysis.

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GC-MS analysis of milk and milk product samples

Gas chromatography- mass spectrophotometry analysis (GC-MS) of fresh milk and milk products for pesticides residues were carried out according to the DFG S9 multi-residue method (Their and Kirchhoff, 1987) using an Agilent Technologies Model 7890A (GC) and Agilent Technologies (MS-5975 C) equipped with a capillary column (HP 5 MS, non-polar inert, 30 m ×0.25 mm diameter×0.25µm film thickness) and an inert XLEI/ CI MSD triple axis detector.

A 1µl aliquot was injected into the GC- MS. The injector was operated in the splitless mode. The column was programmed to operate from 80-160 ^{0}C (10 ^{0}C/min, hold 1 min), 160-250 ^{0}C (6 ^{0}C/min, hold 1 min) and 250-300 ^{0}C (10 ^{0}C/min, hold 5 min). The temperatures of MS Heater were 230-250 ^{0}C for MS source and 150-200 ^{0}C for MS quad. The carrier gas was Helium with a run time of 36 min.

Statistical analysis

Summary statistics (mean and standard deviation) were computed using Minitab 17.0 statistical software.

RESULTS AND DISCUSSION

Method validation

The fresh milk samples and dairy products were analyzed for the presence of Prothiofos, Diazinon, Chlorpyrifos, Oxyfluorfen, Profenofos, Chlorothalonil, Fipronil, Penthoate, Dimethoate, Tebuconazole and Deltamethrin using GC-MS. Method validation is the process of proving that an analytical method is acceptable for its intended purpose. Of the eleven pesticides analyzed, seven were linear across the calibration range with the correlation coefficient of above 0.99. Mean recovery range of milk samples were varied from 80.2 to 100.1% with a relative standard deviation ranged from 7.2-29.1%, indicating an acceptable level of recovery of pesticide residues. Mean LOD and LOQ values were 0.00094 and 0.00290 respectively (Table 1).

Table 1: LOD and LOQ values for pesticides

Pesticide	LOD (ppm)	LOQ (ppm)
Dimethoate	0.003992	0.013307
Chlorothalonil	0.003600	0.012100
Phenthoate	0.000852	0.002840
Profenafos	0.000623	0.002076
Diazinon	0.000020	0.000060

Oxyfluorfen	0.000153	0.000510
Deltamethrin	0.000105	0.000350
Tebuconazole	0.000251	0.000837
Fipronil	0.000796	0.000238
Chlorpyrifos	0.000030	0.000090
Prothiofos	0.000015	0.000050

Pesticide residue levels detected in milk and dairy products

The results obtained from the analysis of 62 fresh cow's milk and dairy products for selected pesticide residues are shown in Table 2.

Table 2: Pesticide residues present in fresh milk and dairy products samples (mg/kg)

Pesticide	Fresh milk	Pasteurized milk	Sterilized milk	Fermented milk	Milk powder
Prothiofos	0.0568± 0.037	0.0225±0	0.016±0	0.012±0	0.005±0
Diazinon	0.0378±0.009	0.0155±0.004	0.0135±0.0007	0.018±0.001	0.0155±0.003
Chlorpyrifos	0.0264±0.004	0.012±0	0.0095±0.0007	0.0085±0.0007	0.014±0.003
Oxyfluorfen	0.0239±0.004	0.0225±0.045	0.0305±0.028	0.01±0.004	0.0065±0.002
Profenofos	0.196±0.099	0.003±0	0.0115±0	0.022±0.004	0.0065±0
Chlorothalonil	0.0652±0.083	0.05±0	0.0475±0	0.014±0	≤ LOD
Fipronil	0.1906±0.188	0.086±0	≤ LOD	0.014±0	0.018±0
Phenthoate	0.1012±0.110	0.0364±0.402	0.0140±0.197	≤ LOD	0.0055±0
Dimethoate	0.1196±0.201	0.0205±0.013	≤ LOD	0.087±0.012	0.0005±0
Tebuconazole	0.062±0.069	≤ LOD	≤ LOD	0.0045±0	0.006±
Deltamethrin	≤ LOD	0.0035±0	≤ LOD	0.0185±0	≤ LOD

Table 2 shows that except Deltamethrin, all other pesticides were identified as residues in fresh milk. Residue levels of Prothiofos (0.0568 mg/kg; MRL=0.05 mg/kg), Diazinon (0.0378 mg/kg; MRL=0.02 mg/kg), Chlorpyrifos (0.0264; MRL=0.02 mg/kg, Profenofos (0.196 mg/kg; MRL=0.01 mg/kg), Fipronil (0.1906 mg/kg; MRL=0.008 mg/kg), Penthoate (0.1012 mg/kg; MRL=0.003 mg/kg), Dimethoate (0.11961 mg/kg; MRL=0.05 mg/kg) and Tebuconazole (0.062 mg/kg; MRL=0.01 mg/kg) present in fresh milk have exceeded the recommended levels set by the WHO (World Health Organization, 2006). Among the pesticides Profenofos (0.196±0.099 mg/kg; MRL=0.01 mg/kg) reported the highest level of residues in fresh cow's milk. The levels of Oxyfluorfen and Chlorothalonil residues observed were below the MRLs of WHO. A similar study in India has identified monocrotophous in the fresh milk samples (Ravichandran *et al.*, 2015). Organophosphorus pesticide residues have been detected in cow's and buffalo's milk products (John *et al.*, 2001). Milk samples analyzed for contaminants revealed that Chlorpyrifos and Diazinon levels presence in the samples were (0.278 mg/kg

and 0.220 mg/kg, respectively) higher than the acceptable limits published by WHO (World Health Organization, 2006). Moreover, the presence of organophosphorus pesticide residues in vegetables has been reported by several researchers; in the Egyptian market (Dogheim *et al.*, 2002) in China (Bai *et al.*, 2006) and in Ghana (Darko and Akoto, 2008).

In general, value added milk products indicated lower levels of pesticide residues compared with the levels detected in fresh milk. Fipronil reported the highest level of residue in pasteurized milk samples (0.086 mg/kg; MRL=0.003 mg/kg) which is higher than the MRL set by WHO. All other pesticide residues present in pasteurized milk had lesser residue levels compared to MRLs of WHO.

Analysis of sterilized milk samples showed that residue levels of Profenofos (0.0115 mg/kg), Chlorothalonil (0.0475 mg/kg) and Phenthoate (0.0140 mg/kg) have exceeded the MRLs of 0.01 mg/kg, 0.02 mg/kg and 0.003 mg/kg respectively. The levels of Prothiofos, Diazinon, Chlorpyrifos and Oxyfluorfen residues were lesser than MRLs of WHO. Moreover, Fipronil, Dimethoate, Tebuconazole and Deltamethrin were below the level of detection in all sterilized milk samples analyzed. It shows that high pressure and temperature used in the manufacturing process (sterilization) has destroyed the residues of Fipronil, Dimethoate and Tebuconazole present in fresh milk.

Fermented milk samples were contaminated with Profenofos (0.0115 mg/kg), Fipronil (0.014 mg/kg) and Dimethoate (0.087 mg/kg) and these values are above the MRLs of 0.01 mg/kg, 0.008 mg/kg and 0.05 mg/kg respectively. The product contained other pesticides at lesser levels than MRLs. However, Penthoate which was present in fresh milk was not detected in fermented dairy products.

Milk powder samples contained two pesticides namely Fipronil (0.01 mg/kg) and Phenthoate (0.0055 mg/kg) at higher levels than MRLs of 0.008 mg/kg and 0.003 mg/kg respectively. Chlorothalonil and Deltamethrin were not detected in milk powder samples. Chlorothalonil has been destroyed by the powder manufacturing process of milk. Other pesticide residues were observed at very low levels in milk powder than MRLs set by WHO.

The source of contaminants could be most probably the feed material used by farmers. In the Nuwara Eliya district, milk farmers feed cows with grasses, crop residues and water which were contaminated due to an extensive use of pesticides in the area (Dilhani *et al.*, 2015, Pathiran *et al.*, 2015). It was observed that farmers use semi intensive (11%) and extensive (15%) raring methods in Nuwara Eliya district (Pathirana *et al.*, 2015). Feed and grasses offered to animals are often contaminated with pesticide residues and after feeding, these residues pass through the body systems (Prassad *et al.*, 2001). When plant materials contaminated with pesticide residues are eaten by herbivores, they are transferred to the food chain, through the main animal originated food sources like milk and meat (Bulut *et al.*, 2011). Moreover, other factors may also contribute to a different degree of contamination that include the application of pesticides on farm animals, environmental contamination and accidental spills (Goordazi *et al.*, 2010).

CONCLUSION

The present study analyzed fresh milk and dairy products collected from the Nuwara Eliya district in Sri Lanka using GC-MS to examine the presence of organophosphorus pesticide residues. Fresh milk contained residues of Prothiofos, Diazinon, Chlorpyrifos, Profenofos, Fipronil, Phenthoate, Dimethoate and Tebuconazole at higher levels than the recommended MRLs of WHO. Compared to other pesticide residues the amount of Fipronil reported the highest residue levels in fresh milk, pasteurized milk and milk powder. Of the sterilized milk samples the highest residue level was reported by Chlorothalonil followed by Oxyfluorfen. Dimethoate content available in fermented milk was the highest among other pesticides. Except for Fipronil, residues of other pesticides present in milk powder showed lower values than MRL. Considering the present level of contamination, preventing the contamination of feedstuffs and ground water sources by pesticides and monitoring the pesticide residue levels of fresh milk are vital to minimize the health risks.

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