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Identification of pesticides residues in different samples of milk

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Abstract

Different pesticides have been used in tropical countries to control agricultural pests. Problem of the presence of pesticides in milk contributes to the serious issue of human health. The identification and quantification of organophosphorous (chlorpyrifos), pyrethroid (bifenthrin, deltamethrin and lambda cyhalothrin), insecticide (imidacloprid) and carbamate (carbofuran) pesticides in cow, buffalo, goat, and camel and sheep milk collected from different localities of Lahore, Pakistan was performed. Milk was monitored by using HPTLC technique for six pesticides residues to determine the degree of contamination. Extraction was performed with ethanol, petroleum ether and sodium oxalate. Extracted milk samples run with pesticides standards. The analysis showed that most of the milk samples were contaminated by the pesticides. Identification of pesticides was done through UV light examination at 254nm and with the standards. Confirmation of pesticides was done through GC-MS. Results showed that 50 % of the milk samples were contaminated with pesticides residues. Deltamethrin was most significantly present in milk samples. Sheep milk samples were mostly contaminated with the pesticides residues. The intake of the pesticide contaminated milk might pose health hazardous to humans in this locality.

Keywords: Pesticides, Residues, HPTLC, UV, GC-MS, Contamination

1. Introduction

Pesticides are used worldwide to increase the production but many persistent residues of pesticides cause problem in environmental contamination and human health. Pakistan is the largest user of pesticides. Several pesticides contain the noxious substances that persist in environment for a long time [8].

Different types of organophosprous and organochlorine have been identified in milk sample. These pesticides can be absorbed by cows through their feed. Problem of the presence of pesticides in milk contributes to the serious issue of human health. Pesticides work to kill the pests or make them ineffective. In the same way, pesticides can affect the unintended individual, such as human.

Pesticide residues have greater impact on human diet so contamination with these residues checked with greater concern in milk and milk products. [11,12].

To control the presence of pesticide residues in fresh milk and milk products is a big issue for producer, consumer and government due to the potential risk. Human milk as well as animal milk is contaminated through the contaminated food. These residues are too much persistent. They accumulate in body fat even in breast milk. These pesticide residues that move in human through milk samples cause different heart problems, endocrine disrupt and cancer.

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Applications of the regular pesticides firstly cause the acute effect then produce the chronic effect and may also cause death [3,7,16].

Pesticides are being used to control the pests in the crops and to increase the quality of the crops. Intensive use of pesticides in agriculture as well as in the community health sector is the major cause of contamination of environment. Accumulation of these dangerous pesticides causes serious health issues in humans. Evidences show the presence of different organochlorine pesticides in crops, human fluids and also in meat. These pesticides also cause little sperm count, increased testicular cancer, different birth defects and different other reproductive defects. A broad range of pesticides are being used in world by farmers because of their wide spectrum activity and greater efficiency. Low cost is also another factor for their use. Because of the harmful effects of organochlorine pesticides these were banned in different time periods in different countries. The main problem of organochlorine pesticides was that they stick within the environment [1,15].

Pakistan is basically an agricultural country. Livestock is playing a vital role in its economy. Almost 30-35 million peoples are engaged with livestock. On the average each family in rural areas has 2-3 cattle's, 5-6 goats and buffalo. People are deriving 30-40% of their income from these animals. Buffalo contributing 68% of milk production, cow 27% and remaining 5% is from sheep's, goats and camel. Buffalo is the major dairy animal of Pakistan and contributing maximum milk production. Pesticides may be classed into two classes' synthetic pesticides and the biological pesticides. Wide spectrum pesticides can kill any species while selected pesticides only kill the selected species for which it has been made. Systemic pesticides are those which are absorbed by the plant. These pesticides come inside the plant circulation. DDT was very famous insecticide and in past its use was very common. 75 % pesticides are being in use by the developed countries. Pesticides save farmer money by killing the unwanted insects or pests in the crops by increasing production yield [2,17].

Pesticides are causing disruptions in the endocrine glands. Hormones play vital role in normal development and pesticides disrupt hormones causing the abnormal development at early stages. Carbamate and organophosphate pesticides block the nerve impulses by reducing the acetyl cholinesterase activity. Most insecticides act on the nervous system of the insect's .Although there is remarkable difference between the nervous system of insects and the mammals but the toxicity mechanism is same in both Different tests are used to check the maximum residue limits of pesticides.

Individual government and the international government set standards for the maximum residue limits. Environmental and the agricultural conditions affect maximum residue limits as these factors are not same in every country [9,11].

In this monitory study milk samples collected from different animals were analyzed for the presence of pesticides residues. The purpose of this study was to check whether milk was contaminated with pesticides or not and what was the limit of contamination with pesticides residues. This study will be helpful for general public and farmers that they should use pesticides with caution.

2. Materials and Methods

A study was done to check six pesticides residues (Table 1) in different milk samples collected from different farms and local areas of Lahore (Table 2). Total of 140 milk samples were analyzed for the presence of pesticides. The residues analysis was done by using High Performance Thin Layer Chromatography (HPTLC) and Gas chromatography-Mass spectrometer (GC-MS) [6].

Information on which six pesticides were selected for analysis is given in table 1.

Selected Animals: Five milk samples were taken from each group buffalo, cow, and goat from each farm. Three milk samples of camel and five milk samples of sheep were taken from each area making total of 115 milk samples.

Sample Collection: A total of 115 milk samples were collected in sterilized 500 ml falcon tubes and immediately transferred to refrigerator to avoid any other environmental contaminations.

Pesticides Standards Collection: Pesticides standards Bifenthrin, Chlorpyrifos, Carbofuran, Deltamethrin were obtained from Ali Akbar Group of Industries and Imidacloprid and Lambda cyhalothrin standards were obtained from Nuclear Institute for Agriculture and Biology (NIAB).

*Sample Extraction***:** 50 ml sample was taken in 250 ml beaker. Then 50 ml of ethanol and 1 gram sodium oxalate and 50 ml of petroleum ether was added in the sample. Shaking was done for two minutes. Centrifugation of the above mixture was done at 15000 rpm for five minutes.

The aqueous layer was separated and was extracted twice with petroleum ether and blown into separator. Water was drained and discarded.

HPTLC Analysis of Pesticide Residues: 0.25mm thick TLC plates were activated at 105 °C for half an hour. Different concentrations of the standards were spotted on TLC plates. A mixture of 45%methanol and 5% ammonia solution was used as mobile phase. Concentration and diameter of the spot were calculated after development of the thin layer chromatography plates. Standards run as reference. Rf values of the standards were calculated. Similarly the extracted milk samples of buffalo, cow, goat, sheep and camel were spotted on preheated TLC plates. Plates were developed in a mixture of 45 % methanol and 5 % ammonia solution which was used as mobile phase. Developed TLC plates were dried and visualized under UV at 254nm.

Detection and Identification of Zones: All the pesticides were not detected through same detection procedure. Some pesticides flourish when observed under UV light source at 254nm. Identification is based on comparison of R_f values for the sample and standard zones and colors obtained with selective-detection reagents. Bifenthrin, Chlorpyrifos, Imidacloprid and Lambda cyhalothrn were developed by spraying o-toludine and potassium iodide. Brown coloured spots appeared. Carbofuran was developed by spraying horse blood serum and acetylthiocholine iodide substrate. Blue color appeared. Deltamethrin was developed by spraying 0.5 % resorcinol in 100 ml of 10 % aqueous NaOH+heat 15 minutes in oven at 100^0C. Pink color spot appeared.

Quantitation of pesticides: Quantitation of pesticides was done by measuring the spot diameters on TLC plate. First of all standard diameters and concentration was calculated. Sample diameters were compared with standard spot diameters and concentration was determined. From this concentration it was determined that how much concentration of pesticide was present in 100 ml of milk and one litre of milk

Results: Milk samples of cow, buffalo, goat, sheep and camel collected from different areas and farms of Lahore were analyzed for six different pesticides (bifenthrin, carbofuran, chlorpyrifos, deltamethrin, and imidacloprid and lambda cyhalothrin). The residue analysis revealed that about 50 % of mil samples were contaminated with the pesticides.From all the pesticides under study the occurrence of the deltamethrin residues was highest followed by carbofuran. 50 percent of the milk samples were contaminated with pesticides residue. Among cow milk samples bifenthrin was detected in 17 cow milk samples, chlorpyrifos was in 12, carbofuran , deltamethrin, and imidacloprid was detected in 16 each and lambda cyhalothrin was detected in 13 milk samples. Among buffalo milk samples bifenthrin was detected in 15 milk samples, chlorpyrifos was in 17, carbofuran was in 14, deltamethrin was in 16, imidacloprid was in 14 and lambda cyhalothrin was in 13 milk samples. Among goat milk samples bifenthrin was detected in 13 milk samples, chlorpyrifos was in 15, carbofuran was in 16, deltamethrin was in 14 and lambda cyhalothrin was detected in 15 goat milk samples. Among sheep milk samples bifenthrin was detected in 13 milk samples, chlorpyrifos was in 12, carbofuran was in 11, deltamethrin was in 16, imidacloprid was in 14 and lambda cyhalothrin was detected in 13 milk samples. Among camel milk samples bifenthrin was detected in 9 milk samples, chlorpyrifos was in 9, carbofuran was in 8, deltamethrin was in 9, imidacloprid was in 6 and lambda cyhalothrin was detected in 7 milk samples. Average pesticide concentration for each pesticide in each animal also been calculated. Concentration of pesticides from each farm in the milk of animals calculated and then average value for each farm determined for each animal milk sample. Similarly for sheep's and camel milk samples area wise average values were calculated. All these average values were shown in tabulated form in table 3.

When pesticides analysis had done every pesticide showed its maximum value in different samples of milk. Following are the highest levels of pesticides residues found in milk samples shown in table 4.

Table 1: Pesticides with molecular formulas and basic classification

Pesticide Name	Molecular Formula	Classification
Bifenthrin	$C_{23}H_{22}ClF_3O_2$	Synthetic Pyrethroid
Carbofuran	$C_{12}H_{15}NO_3$	Carbamate
Chlorpyrifos	$C_7H_{16}ClN_3O_2S_2$.	Organophosphate
Deltamethrin	$C_{22}H_{19}Br_2NO_3$	Synthetic Pyrethroid
Imidacloprid	$C_9H_{10}ClN_5O_2$	Insecticide
Lambda-cyhalothrin	$C_{23}H_{19}ClF_3NO_3$	Pyrethroid

Table 2: Selected animals and sampling areas

Animals Selected	Sampling Areas
Buffalo **Cow** **Goat**	UVAS Pattoki Campus (Farm 1) Mach Dairy Farm (Fram 2) Taza Dairy Farm (Farm 3) Al-Mashraq Dairies (Farm 4) Barki Dairy Farm (Farm 5)
Sheep **Camel**	Shahdra Garhi Shahoo Begum Kot China Sacheme Gujjar Pura Saggian

Table 3: Quantitative results for collected milk samples

Areas	Animals	Pesticides concentration in mg/L					
		Bifenthrin	**Chlorpyrifos**	**Carbofuran**	**Deltamethrin**	**Imidacloprid**	**Lambda cyhalothrin**
Farm 1	**Cow**	0.15	0.11	0.18	0.057	0.100	0.019
	Buffalo	0.13	0.13	0.12	0.061	0.12	0.120
	Goat	0.12	0.04	0.02	0.070	0.140	0.034
Farm 2	**Cow**	0.13	0.002	0.10	0.80	0.10	0.80
	Buffalo	0.17	0.19	0.120	0.75	0.99	0.10
	Goat	0.11	0.09	0.09	0.60	0.80	0.067
Farm 3	**Cow**	0.19	0.109	0.840	0.744	0.310	0.10
	Buffalo	0.17	0.740	0.601	0.681	0.44	0.71
	Goat	0.091	0.761	0.081	0.091	0.041	0.044
Farm 4	**Cow**	0.61	0.23	0.33	0.53	0.41	0.62
	Buffalo	0.52	0.04	0.09	0.10	0.21	0.68
	Goat	0.52	0.04	0.09	0.10	0.21	0.68
Farm 5	**Cow**	0.733	0.412	0.10	0.18	0.170	0.120
	Buffalo	0.02	0.80	0.20	0.69	0.111	0.510
	Goat	0.19	0.081	0.740	0.601	0.233	0.423
Shahdra	**Sheep**	0.10	0.071	0.65	0.72	0.45	0.76
	Camel	0.72	0.42	0.55	0.72	0.16	0.19
Garhi Shahoo	**Sheep**	0.20	0.69	0.111	0.510	0.30	0.40
	Camel	0.43	0.10	0.010	0.16	0.14	0.62
Begum kot	**Sheep**	0.41	0.37	0.55	0.80	0.71	0.744
	Camel	0.11	0.21	0.09	0.51	0.044	0.10
China scheme gujjar pura	**Sheep**	0.744	0.569	0.623	0.401	0.021	0.10
	Camel	0.35	0.21	0.49	0.38	0.68	0.721
Saggian	**Sheep**	0.571	0.482	0.491	0.339	0.191	0.371
	Camel	0.69	0.75	0.389	0.20	0.10	0.320

Table 4: Maximum pesticides residue level in milk samples

Animals	Pesticides					
	Bifenthrin	**Chlorpyrifos**	**carbofuran**	**deltamethrin**	**imidacloprid**	**Lambda cyhalothrin**
Buffalo	0.52	0.80	0.60	0.75	0.99	0.71
Cow	0.733	0.41	0.84	0.80	0.41	0.80
Goat	0.71	0.76	0.74	0.71	0.80	0.69
Sheep	0.74	0.69	0.65	0.80	0.71	0.76
Camel	0.72	0.75	0.55	0.72	0.68	0.72

Statistical Analysis: The quantitative data on pesticide concentration in milk samples was subjected to two-way analysis of variance (ANOVA) and F value for pesticide concentration was calculated. Statistical analysis showed that deltamethrin was most significantly present in milk samples among pesticides. Whereas sheep milk samples were found to be mostly contaminated with pesticides.

Discussion: The major source of contamination of dairy products by different hazardous pesticides is the presence of their residues in animal feed stuffs. Other factors that may also contribute to this sort of contamination include the application of pesticides on farm animals, environmental contamination and accidental spills [4]. Milk contamination with the pesticides residues can be controlled by preventing the contamination of feedstuffs. In this monitory study residues of different pesticides were determined by HPTLC analysis which showed high levels of deltamethrin, carbofuran, chlorpyrifos, imidacloprid, bifenthrin and lambda cyhalothrin residues in milk samples exceeding MRL's. The percentage of the milk samples contaminated with pyrethroid pesticide residues in present study was more than as compared to reported by Muccio et al. (1999) [10] who analyzed commercial homogenized milk for the presence of pyrethroid pesticide residues. The average level found was 0.04-0.41 mg/kg. But this study showed that the level of pyrethroid pesticide residue was 0.02-0.80 mg/kg.

Organophosphate pesticides residues were present in collected milk samples in the present study. Organophosphate and organophosphate pesticides residues were also detected in the milk samples collected by Indraningsih et al. (2004) [5]. In the present study no organochlorine pesticide residue was detected as it has been banned because of its lethal effects on humans.

A similar study was done on dairy sheep's and goat milk and feed samples for the detection of pesticides residues in Greece by Tsiplakou et al. (2010) [14]. Endosulfan was the main pesticide residue which was detected in the feed samples with the average of 0.10 mg/kg and this level was lower than the MRL level. In sheep and goat milk samples no pesticides were detected and this milk was considered to be safe for human use. In the present study sheeps milk samples were mostly contaminated with the pesticides residues. 50 % of goat milk samples were also contaminated with pesticides residues. In sheep milk samples the highest level of deltamethrin found was 0.80 mg/kg and in the goat milk samples imidacloprid was at 0.80 mg/kg.

13 milk samples were analyzed by HPTLC by Prof. Jackson Ombui (2013) [13] for the presence of organophosphate and pyrethroid pesticides residues. 73 % of Deltamethrin was present in milk samples. It means mostly milk samples were contaminated with deltamethrin pesticide residue. Similarly in the present study statistical analysis showed that deltamethrin was present most significantly in the milk samples as compared to other pesticide residues.

Conclusion: In this study 140 milk samples were collected from buffalo, cow, goat, sheep and camel. These milk samples were analyzed through HPTLC for the presence of six pesticides residues (bifenthrin, chlorpyrifos, carbofuran, deltamethrin, imidacloprid and lambda cyhalothrin). The study findings revealed that 50 % of the collected milk samples were contaminated with the pesticides residues. The method used HPTLC can be used for the identification and separation of pesticides residues in milk samples. The findings of the study might help in extending awareness in dairy farmers and local people about pesticides and their hazardous effects on humans.

Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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