

Pesticide residues composition of milk samples of female farm workers

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Abstract

The experiment was conducted to monitor the pesticide residues in milk samples of rural female farm workers engaged in cotton, okra, chilies picking, berseem and wheat harvesting activities in Sindh province of Pakistan. A total of 80 samples were collected in this study 20 samples were randomly collected from Cotton Pickers, Okra Pickers, Chilies Pickers, Berseem and wheat harvesters. Collected samples were stored in pre-sterilized glass bottles, sealed carefully and immediately placed in ice box for transportation to the laboratory at the Institute of Food Science and Technology, SAU, Tandojam and stored in freezer at -20°C until further analysis. The samples were extracted and cleaned up by QuEChERS method as given in AOAC (2007) and analyzed through GC-MS.

The Endosulfan, Chlorpyrifos, Lambda Cyhalothrin and Profenofos were found as major milk pollutants. Endosulfan and DDT was found in all milk samples of female vegetable pickers collected from Tando Allahyar, Tando Muhammad Khan, Umer Kot and Hyderabad. Lambda Cyhalothrin, Chlorpyrifos and Profenofos were observed highest in the samples collected from Tando Muhammad Khan district i.e. up to 85, 55 and 80% respectively. Similar but

little less trend was also observed in other three districts. DDT was also recorded major milk pollutant in almost all the studied districts. Thus, it is concluded from the study that samples collected from female farm workers contained the pesticide residues and the lactating mothers may transfer these pesticides to their offspring's during pregnancy and lactation. Therefore, there is dire need of Integrated Pest Management and Farmers Field School program to adapt as a part of essential component of Good Agriculture Practices in Pakistan.

Key words:

Milk; QuEChERS; GC-MS; pesticide residues; female farm workers

1. Introduction

Use of pesticides is not uncommon to protect the crop from insect pests and diseases and get higher yields. Pesticides are poisons and used to kill insects and pests (Wassemann, 1972; Ahrens, 1994). Due to indiscriminate and injudicious use of pesticides, many incidents have taken place affecting not only human health but soils crops and the environment as well so much so even the women breast milk affection is also a matter of great concern. Persistence of

the residues of pesticides has been considered most hazardous among all other pesticides released every year by human activity in the farm fields. These are causing an array of adverse effects because of toxic nature resulting in birth defects, diseases and death among human and the animals.

Female farm workers who are actively involved in various agricultural farm practices particularly in Sindh province of Pakistan play a significant role in vegetable production and other agricultural activities including cottage enterprenual practices in the rural areas.

Presence of toxic residues of pesticides have been reported in various environmental component because of its wide spread use on farm fields (Kumari and Kathpal 2008; Wang *et al.*, 2008) and residues of these pesticides find their way into the human body through water, environment and food. It is therefore the pesticide residue analysis in food, water, vegetables, fruits and soil have become an essential requirement for producers, food quality control authorities and the consumers.

Women have a particular susceptibility to pesticides due to their physiological characteristics, lifestyle, and behavior. Farm women workers are always at a greater risk of accumulated exposure because of long working hours from an early age and frequent exposures (at work and in domestic settings) with potential exposition to pesticides eating pest contaminated diet, washing of pest exposed clothing, drinking contaminated water, and intensive use of a multitude of pesticides in agriculture (Rother, 2000). In recent analytical studies on cancer among 146,000 California Hispanic women farmers as compared with general Hispanic population, it was confirmed that farm women workers were more likely to develop various types of cervical cancer. It

was noted that 63% of them were affected by cervical cancer, 68% by uterine, 70% by stomach cancer and 59% by instances of leukemia as compared to none farm activity related population.

Studies have also documented an increased incidence of delayed pregnancy, still births, and miscarriages among women working in farms and wives of men employed in pesticide spraying and mixing in agricultural-food industries (Ransom, 2002). It has also been reported that the acute effects of pesticide spray on farm women were affected with nausea, muscular pain, sore eyes, difficulty in breathing, itching, dizziness, skin burns, sneezing, blisters, nails color change (Ransom, 2002; Jacobs and Dinham, 2003). The study therefore was planned to monitor pesticide residues in breast milk samples of female farmers of Sindh to determine the level of affection caused by the pesticides.

2. Materials and Methods

2.1. Sample collection

Milk samples were collected from female vegetable pickers working in the fields of chilies, Barseem/ wheat, cotton and okra from Umer Kot, Hyderabad, Tando Muhammad Khan and Tando Allahyar districts, respectively. The sampling of milk was done at the highest peak of the production stage of vegetable crops. Milk samples were obtained from vegetable pickers engaged in picking activities in plots receiving the traditional agricultural practices. 80 milk samples were collected from different vegetable fields and included in this study (Table 1). Collected samples were stored in pre-sterilized glass bottles, sealed carefully and immediately placed in ice boxes for transportation to the laboratory at the Institute of Food Sciences and Technology, Sindh Agriculture University

Tandojam and stored in freezer at -20°C until further analysis.

Table 1. Milk samples collected from vegetable pickers

Name of Samples	Vegetable pickers
Cotton Pickers	20
Okra Pickers	20
Chilies Pickers	20
Berseem and wheat harvesters	20
Total	80

2.2. Chemicals and reagents

Acetonitrile, anhydrous magnesium sulphate, sodium chloride, PSA (primary Secondary Amine), and dispersive SPE (dSPE) tubes were purchased from Aldrich-Sigma, USA. Double distilled water was obtained in the laboratory using Fisons' apparatus (Ecublens, Switzerland).

Pesticide standards Endosulfan, Chlorpyrifos, Lambda Cyhalothrin and Profenofos of 99.9% purity were purchased from Aldrich-Sigma (USA). Standard solutions (1mgml^{-1}) were prepared by dissolving the GC-amenable pesticides in n-hexane. These solutions were further diluted to have working solutions for spiking ($1\text{ }\mu\text{gml}^{-1}$)/ preparation of standard curves.

2.3. Residue extraction and cleanup

Pesticide residues from milk samples were extracted by QuEChERS method. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method (first reported by Anastassiades *et al.* 2003) has been endorsed and approached by Association of Office Analytical Chemists as official method (AOAC, 2007) for extracting pesticides from food samples. Because of its simplicity and effectiveness the method became very common for pesticide analysis in fatty food samples (Lehotay and Maotovska, 2005). This method is known as a multiclass, multi-residue method (MRM) for sample preparation, cleanup and for the analysis of multiple residues.

Milk samples were prepared and extracted by QuEChERS Method as described by

Brondi *et al.* (2009). In 50ml poly propyl centrifuge tube name ECMSSC50CT 10ml milk, 10 mL of MeCN, 6 g of anhydrous MgSO_4 and 1 g of NaCl were added and was shaken well. Next the tube was centrifuged at 3000 rpm for 1 min and the supernatant was transferred to second centrifuge tube (UCT ECMPS1815CT) which is 15 ml centrifuge tube with 900 mg anhydrous magnesium sulfate, 300mg PSA and 150mg end capped C18. It was then mixed vigorously for one minute, centrifuged again for one minute at 3700 rpm. The extract was filtered and the filtrate was placed in 15 mL screw capped glass vial for analysis by GC-MS.

Magnesium sulphate was added to aid in partitioning and acetonitrile was used as a solvent because it causes maximum extraction of organic analytes (pesticide residues) without extracting lipophilic interfering material. Salts such as MgSO_4 and NaCl and n-hexane during shaking induce separation between water and acetonitrile and bind water to extract pesticide analytes into n-hexane phase, thus causing maximum recovery of pesticides from milk. PSA (Primary secondary amine) and carbon sorbent removed organic acids, pigments, planar compounds, sugars and sterols.

2.4. Instrumental analysis

GC-MS examination was performed with an Agilent Technologies 6890n system GC instrument furnished with a 5975 inert MSD in mode of Electron Impact ionization

(EI), and programmed split/split less injector model Agilent 7683, HP-5MS 5% Phenyl Methyl Siloxane capillary column (30 m × 0.25 mm × i.d., 0.25µm film thickness). The mobile gas utilized was Helium (with up to 99.9993% purity). The working conditions were as per the following: injector port temperature 280 °C, pressure 12.75 psi, injection volume 2µl in split less mode, helium as carrier gas at a flow rate of 17.1 mlmin⁻¹; oven temperature program, 80 °C (2 min), increased at 30 °C/min to 180 °C, then increased to 200 °C at 1.5 °C/min and finally increased at 20 °C/min to 280°C and held for 8 min, solvent delay, 4.00 min. The total analysis time is 31.00 min. The mass spectrometer was worked in electron impact ionization mode (ionizing energy = 70 eV) examining from m/z 80 to 550. The ion source (EI) and quadrupole temperatures were 230 and 150 °C, individually.

Pesticides were qualitatively detected utilizing RTL screener programming as a part of consolidation with Agilent RTL pesticide library and quantitatively measured by setting up the standard bends of different concentrations of particular pure standards.

2.5. Residue recovery efficacy

The efficacy of pesticide residual recovery was determined by the blank samples fortified with 1.0 mgkg⁻¹ of pesticide standard and then subjected to extraction. Clean-up and estimation procedures are those as for unknown samples. Percent recoveries were reproducible (85–100%) and the residues calculations for unknowns were done accordingly.

3. Results

3.1. Potential exposure of pickers to pesticides

Frequencies of farm pickers' practices leading to potential exposure to pesticides are summarized in table 2. Although cotton and vegetable is the main activity, majority was in weeding activity also. Weeded plants are generally used as fodder. All pickers reported that they were not using hand gloves because they were able to pick more produce without gloves and wages were tagged with the quantity of the produce picked. Most of the pickers (83%) reported picking activity within 48 hours of pesticide application whereas all the pickers avoided picking activities during first day of spray. Hence cotton, okra and chilies pickers were prone to more exposure to pesticides than barseem and wheat pickers.

Table 2. Frequencies of farm pickers' practices leading to potential exposure to pesticides.

Practice	Frequency (%)			
	Cotton pickers (N = 20)	Okra pickers (N = 20)	Chilies pickers (N = 20)	Berseem and wheat harvesters (N = 20)
Farm Practices that lead to pesticide exposure				
Vegetable picking and harvesting	100	100	100	100
Weeding in Vegetables	66	50	20	NIL
Kind of protective cover				
No or only partial protective covering (short sleeve, barefooted, without hand gloves, etc)	100	100	100	100
Full protective covering (long	NIL	NIL	NIL	NIL

sleeves, hand gloves, rubber boots, etc)				
Farm picker re-entry periods				
Less than 48 h	83	NIL	49	N/A
From 48 to 72 h	17	33	40	N/A
More than 72 h	NIL	67	11	N/A

3.2. Clinical manifestations in farm pickers

Berseem and wheat harvesters were asymptomatic and only few complained about general weakness which may be due to malnutrition. However, Cotton pickers, chilies pickers and okra pickers were found with the symptoms including headache, dizziness, eye irritation, general weakness and fatigue, itching, digestive system (indigestion/vomiting), and respiratory

system (coughing/ breathing problems). The symptoms of the pickers are shown in Table 3. Most affected were chilies pickers and the most frequently ailment reported by them were headache with 60%, followed by dizziness with 50%, eye irritation and itching 30% each, digestive system and weakness 20% each and respiratory system 10%. Similar findings were also reported by other researchers (Mehboob *et al.*, 2012; Longnecker *et al.*, 1997).

Table 3. The most frequent ailments reported by the farm pickers

Ailment reported	Cotton pickers	Chilies pickers	Okra pickers	Berseem and wheat harvesters
Headache	40	60	50	-
Dizziness	10	50	40	-
Eye irritation	-	30	-	-
Digestive system	20	20	10	-
Respiratory system	30	10	-	-
Itching	-	30	10	-
Others: General weakness & fatigue	30	20	40	10

The lower arms, hands and feet were the mostly unprotected body parts during picking activity. Although pickers reported that they washed hands for eating meals. They also changed their work clothes toward the end of the workday. Washing of clothes used in fields along with the clothes of rest of the family was common practice which may lead to the spread of pesticide compounds in all family members. Because of financial constraints combined with unavailability of qualified doctors in rural areas, they took home remedies or they medicated themselves to treat the ailments.

3.3. Pesticide residue analysis

3.3.1. Pesticide residue analysis in milk samples of lactating farmer women from cotton pickers of district Tando Muhammad Khan

Residues of four pesticides namely DDT, Endosulfan, Profenofos, Chlorpyrifos and Lambda Cyhalothrin were detected in milk samples of vegetable pickers of cotton pickers (Table 4). Pickers had high endosulfan residues with mean value of 0.0023 mgL⁻¹ with highest value of 0.0045 mgL⁻¹. While, the second most prevalent was profenofos detected in vegetable pickers

with mean level of 0.036 mgL^{-1} with higher level of 0.048 mgL^{-1} , lambda cyhalothrin was detected in vegetable pickers with mean level of 0.011 mgL^{-1} and higher level of

0.041 mgL^{-1} . The chlorpyrifos was detected in milk samples up to 55% pickers, respectively.

Table 4. Mean and range of pesticide residues (mgL^{-1}) in whole milk samples collected from cotton pickers of district Tando Muhammad Khan

Pesticide	TL*	Mean	Range**	% positive
ΣDDT***	(0.02 ppm)	0.01	0.001-0.036	55
Endosulfan	(0.004ppm)	0.0023	0.001-0.0045	100
Chlorpyrifos	(0.02ppm)	0.016	0.001-0.04	55
Profenofos	(0.01ppm)	0.036	0.001-0.048	80
Lambda Cyhalothrin	(0.03ppm)	0.011	0.001-0.041	85

*TL= Tolerance limit

*Range values for positive samples

***ΣDDT = sum of p,p-DDT, p,p-DDD, p,p-DDE, o,p-DDT, op-DDD, op-DDE

3.3.2. Pesticide residue analysis in milk samples of lactating farmer women from chilies pickers of district Umer Kot

Mean and range of pesticide residues (mgL^{-1}) in milk samples collected from 20 vegetable pickers of chilies are given in

Table 5. Endosulfan and DDT was detected in 65 and 100% vegetable pickers, respectively. Lambda Cyhalothrin was the next most frequently detected pesticide and it's was found in 65% pickers. On the other hand chlorpyrifos and profenofos were found in 40 and 55% pickers, respectively.

Table 5. Mean and range of pesticide residues (mgL^{-1}) in whole milk samples collected from chilies pickers of district Umer Kot

Pesticide	TL*	Mean	Range**	% positive
ΣDDT***	(0.02 ppm)	0.017	0.01-0.026	65
Endosulfan	(0.004ppm)	0.005	0.0001-0.046	100
Chlorpyrifos	(0.02ppm)	0.011	0.0002-0.15	40
Profenofos	(0.01ppm)	0.012	0.001-0.036	55
Lambda Cyhalothrin	(0.03ppm)	0.012	0.001-0.069	65

*TL= Tolerance limit

*Range values for positive samples

***ΣDDT = sum of p,p-DDT, p,p-DDD, p,p-DDE, o,p-DDT, op-DDD, op-DDE

3.3.3. Pesticide residue analysis in milk samples of lactating farmer women from okra pickers of district Tando Allahyar

Pesticide residues detected in vegetable pickers are shown in Table 6. Endosulfan

and DDT was found in 60 and 100% of pickers, respectively (mean 0.005 and 0.013 mgL^{-1} , respectively) followed by lambda cyhalothrin up to 50% (mean of 0.017 mgL^{-1}). Profenofos was found up to 30% whereas

chlorpyrifos were present in 45% pickers only.

Table 6. Mean and range of pesticide residues (mgL⁻¹) in whole milk samples collected from okra pickers of district Tando Allahyar

Pesticide	TL*	Mean	Range**	% positive
ΣDDT***	(0.02 ppm)	0.013	0-0.048	60
Endosulfan	(0.004ppm)	0.005	0-0.046	100
Chloropyrifos	(0.02ppm)	0.012	0-0.048	45
Profenofos	(0.01ppm)	0.007	0-0.024	30
Lambda Cyhalothrin	(0.03ppm)	0.017	0-0.036	50

*TL= Tolerance limit

*Range values for positive samples

***ΣDDT = sum of p,p-DDT, p,p-DDD, p,p-DDE, o,p-DDT, op-DDD, op-DDE

3.3.4. Pesticide residue analysis in milk samples of lactating farmer women from barseem and wheat pickers of district Hyderabad

Pesticide residues detected in barseem and wheat harvesters are shown in Table 7. Endosulfan and DDT was found in 50 and 40%, respectively of pickers (mean 0.0026 and 0.002 mgL⁻¹, respectively) followed by profenofos up to 25% whereas chlorpyrifos were present in 30% pickers only.

Table 7. Mean and range of pesticide residues (mgL⁻¹) in whole milk samples collected from berseem and wheat pickers of district Hyderabad

Pesticide	TL*	Mean	Range**	% positive
ΣDDT***	(0.02 ppm)	0.002	0.001- 0.003	40
Endosulfan	(0.004ppm)	0.0026	0.001-0.046	50
Chloropyrifos	(0.02ppm)	0.002	0.001-0.005	30
Profenofos	(0.01ppm)	0.005	0.002-0.007	25

*TL= Tolerance limit

*Range values for positive samples

***ΣDDT = sum of p,p-DDT, p,p-DDD, p,p-DDE, o,p-DDT, op-DDD, op-DDE

4. Discussion

The pesticide usage is increasing day by day and now has become mandatory to increase food production to feed the growing population though out the globe. It is stated by John *et al.* (2001) that the plants sprayed with pesticide gives increased agriculture produce to a large amount. Though the increase in use of pesticides has increased the nation's economy, but it is also affecting environment as well as severe health concern both for male and female. According to Khaniki (2007) and Tsiplakou

et al. (2010) that various pesticides have been found as contributing factors for the Alzheimer's disease, Parkinsonism and heart diseases. The presence of pesticide residues in fodder and feed of dairy animals may assimilate in its body systems (Prasad and Chhabra, 2001), leads to milk and meat contamination (Hernandez *et al.*, 2010) thereby affecting human beings upon consumption.

Women milk is a route for elimination of xenobiotics and also a source of

contamination for the baby on breast feed (Campoya *et al.*, 2001). The organochlorine concentration in women milk mainly depends on its accumulation in the fatty tissues of mother and subsequent mobilization. The exposure of offspring's to environmental contaminants through mother's milk has drawn the attention for the safety and protection of babies (Matsuda *et al.*, 1978; McLahlan, 1980; Quinsey *et al.*, 1996). The daily intake of a breast-feeding child (per kilogram body weight) estimated to be fifty folds that of an adult, so that breast-feeding baby (First 6 months) accumulates much amount of pesticides as an adult does in 25 years. Thus, a load of pesticide molecules passed from one generation to another through breast feeding (Nunes and Tajara, 1998) could well be imagined. The risk of pesticide transfer is not only limited to lactation but, every living being in the locality can similarly be affected by pesticides. Wong and Lee (1995) and Waliszewski *et al.* (1998) therefore, expressed that Bio-health controls must be established for the animals and women whose milk provides infant formulas (Wong and Lee, 1995, 1998).

Residues in milk are one of the most widely used food stuffs containing lipids which could be a quantitative and qualitative index for the presence of these toxins in bodies. After ingestion, these lipophilic pesticides get absorbed from the intestine into the general circulation. Highly lipid soluble pesticides tend to concentrate in tissues with higher lipid contents including adipose tissue, brain, liver, kidneys and in milk (Tsiplakou *et al.*, 2010; Kampire *et al.*, 2011). Fytianos *et al.* (1985) and Abou Donia *et al.* (2010) also confirmed these findings and reported the accumulation of a high level of pesticide residues in larger fat milk as compared to low fat milk.

The presence of the residues of DDT, endosulfan, profenofos, chlorpyrifos and lambda cyhalothrin have been found as major milk pollutant in this study and deserve immediate attention. Muhammad *et al.* (2012) reported 250 ppb (or 62 times above MRLs) endosulfan residues in milk from Faisalabad, Punjab, Pakistan. Endosulfan is still being used in Pakistan where as same is banned in many countries including India due to its low cost, wide spectrum and potent effects against hazardous insect's pests. The main health hazards associated with exposure to these compounds are respiratory diseases, diarrhea, nonfunctioning of the reproductive system, abdominal pains and hypertension pre/post natal damage, etc (Akhtar *et al.*, 2009; PNNAP, 2012). Endocrine disruptors including endosulfan are also known to produce adverse effects by antagonizing or imitating the natural hormones inside body. It has also been reported that their low-dose, long-term exposure is increasingly linked to human health problems (i.e., diminished intelligence, hormone disruption, immune suppression, cancer and reproductive abnormalities (Akhtar *et al.*, 2009). The results published in the magazine "Down to Earth" also reflected that very high levels of Endosulfan were found in samples of cashew, cow's milk, human milk, spices, animal tissues, human blood, vegetables, soil and cashew leaves (Joshi, 2001).

Dichlorodiphenyltrichloroethane (DDT) is a potent contact insecticide. Although the use of DDT has been banned in agriculture since 1993 but it is still in use. It could therefore be the reason of the presence of DDT and its analogues in the environment. p,p'-DDT was detected in almost all samples collected from Tando Muhammad Khan, Tando Allahyar, Umer Kot and Hyderabad, similar readings are also reported (Ashnagar *et al.*, 2009). Total DDT (sum of pp'-DDE + pp'-DDD + op'-DDT + pp'-DDT) was detected

in all of samples collected. Since DDT is known to undergo metabolic conversion and dehydrochlorination presence of metabolites of DDT *i.e.* DDD and DDE encountered in this study might be due to such metabolic processes. This analysis indicates that DDT and Endosulfan are the major contaminants in different parts of Sindh.

5. Conclusions

It was concluded that high level residues of DDT, Endosulfan, profenofos, lambda cyhalothrin, and chlorpyrifos were detected in the milk samples of farm pickers which manifested various ailments in the pickers. This is indicative of occupational exposure. Chilies, cotton, okra women pickers from districts Umer Kot, Tando Muhammad Khan, Tando Allahyar were highly exposed by pesticide residues as well as their health were highly affected as compared with the barseem and wheat harvesters of district Hyderabad. Therefore it is highly advisable to abandon the use of such synthetic pesticides. Besides FFS, IPM technology may be transferred to the growers through other means such as electronic and print media.

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