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HAMID, Almas; YAQUB, Ghazala; AHMED, Sajid Rashid; AZIZ, Nida

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Assessment of human health risk associated with the presence of pesticides in chicken eggs

Almas HAMID[1]*, Ghazala YAQUB[1], Sajid Rashid AHMED[2], Nida AZIZ[1]

Abstract

The presence of pesticides in the environment is highly toxic to environment and human health. Aim of the study was determination, quantification and assessment of associated health risk due to presence of pesticide residues in chicken eggs using high pressure liquid chromatography. HPLC method was successfully employed and validated. From collected samples pesticides were extracted in presence of petroleum ether and acetonitrile. Bifenthrin and Difenoconazole residues were found in all samples with different concentration exceeding maximum residue limits (MRL) of Codex Alimentarius Commission. However imidacloprid was not detected in any sample. Concentration of bifenthrin in house egg samples ranged from 0.256206 to 4.112387 mg/kg while in poultry farm samples it varied from 1.5862 to 5.80796 mg/kg. Difenoconazole was found in concentration of 0.02835 mg/kg, 1.7668 mg/kg, 3.7205 mg/kg, 21.8937 mg/kg 21.9835 mg/kg, 19.26407 mg/kg in samples collected from houses while and in poultry farm samples its detected concentration was 10.939 mg/kg, 12.3296 mg/kg, 29.3617 mg/kg, 18.6116 mg/kg, 40.0523 mg/kg and 19.2335 mg/kg. Concentrations of both pesticides Bifenthrin and Difenoconazole exceeded the MRLs (0.05 mg/kg). Health risk index surpassed 1 (the cut off value) for Difenoconazole in seven samples while for Bifenthrin values were less than 1, indicating the possibility of potential medium to long term health risk associated with ingestion of contaminated eggs.

Keywords: Organochlorine pesticides; HPLC; chicken eggs; poultry samples; maximum residue limits.

Practical Application: The study holds significant importance in order to ensure that contaminant exposure via food intake does not exceed acceptable levels. There is a lack of data regarding levels of pesticides in food. In developing countries very few studies are available on the quantification and estimation of health risk due to pesticide residues in chicken eggs. The present study was thus under taken in order quantify selected pesticides present in chicken eggs by highly sensitive and selective method of High Performance Liquid Chromatography.

1 Introduction

Hazardous substances including persistent, bio-accumulative and toxic substances (PBTs), are chemicals which do not degrade easily in the environment. They typically accumulate in fatty tissues and are slowly metabolized often resulting in increased concentration through the food chain. Pesticides have also been used for breeding animals mainly to protect them from infestation of pests. The main source of human exposure to these pesticides is ingestion of animal and plant products that have been treated with persistent pesticides. Due to health concerns related to pesticides contaminated plants and foodstuff, European Union established Maximum Residue Limits (MRLs) for pesticides found in food of animal origin such as meat products, eggs and poultry products (Food and Agricultural Program of the United Nation, 2015).

Poultry meat and eggs forms an important constituent of diet world over, owing to its relatively lower cost and superior nutritional value. The poultry sector in developing countries like Pakistan is an important and significant sector of agriculture, contributing a share of 1.3% to the national GDP (Hussain et al., 2015), 6.4% in agriculture and 22.5% to livestock and a considerable share in provision of direct and indirect employment and income to an estimated 1.5 million people. Chlorinated pesticides and (halogenated) environmental contaminants can enter laying hens in a variety of ways. The most important way is via the feed (including soil intake). However, direct application on the animal and contact with the skin as well as inhalation via the airways should not be ruled out. Contamination of food sources, crops as well as food of animal origin, has emerged as a serious threat to food security and human health, owing mainly to the rise in pollution levels. Increased population, rapid industrialization and urbanization have created pollution issues with contaminants accumulation along the food chain. Organochlorine (OC) pesticides are highly stable, toxic and have high affinity for lipids. Their use was banned in the early 1980s because their adverse health effects including cancer, hormonal disruption and reduction of bone density. These pesticides are still being used in developing countries including India mainly for malaria control (Koc & Karakus, 2011) Organochlorine pesticides cause acute and chronic health effects, irritation of the skin and eyes, affecting nervous system, mimicking hormones causing reproductive problems, cancer, neurological effects, birth defects, fetal death and neurodevelopment disorders etc (Matial et al., 2003; Aulakh et al., 2006). Literature suggests that

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[1] *Department of Environmental Sciences, Kinnaird College for Women, Lahore, Pakistan*
[2] *College of Earth and Environmental Sciences, University of the Punjab, Quaid-e-Azam Campus, Pakistan*
*Corresponding author: almas209@yahoo.com

daily intake of pesticides by chicken leads to excretion of these residues in eggs in varying quantities depending on the type of pesticide (Hashemy-Tonkabony & Mosstofian, 1978). Therefore, the awareness and need for regular screening of these foodstuffs is necessary and is in interest of both international trade and consumers. Determination of pesticide residues can be done using a variety of analytical techniques. The conventional methods to obtain analytes, including organic pesticides, involve several steps beginning from homogenization followed by procedures for liquid-liquid extraction. Prior to chromatographic separation, clean-up steps and extract purification for the removal of co-extractants take place. These methods require large amount of matrix and the consumption of solvent is also high. Recent developments in anal**y**tical methods involve use of less hazardous organic solvents and minimum evaporation steps. In previous studies, HPLC, GC and GC/MS were successfully employed for detection of pesticides in various environmental matrices (Khan et al., 2015; Hamid et al., 2016). Yet still **t**here is a lack of data regarding levels of pesticides in food. In Pakistan very few studies are available on the pesticide residues in chicken eggs. The present study was thus under taken in order quantify selected pesticides present in chicken eggs by High Performance Liquid Chromatography with method modification. The study holds significant importance in order to ensure that contaminant exposure via food intake does not exceed acceptable levels.

2 Materials and methods

The current study was carried out to determine the concentration of pesticides in chicken eggs. Following methodology was adopted for this purpose. All solvents, reagents i.e., anhydrous sodium sulphate, petroleum ether, nitrogen, acetonitrile and methanol and pesticide standards of analytical grade were used for analysis.

2.1 Sampling

A total of 72 egg samples were collected. All collected chicken egg samples were locally produced. Eggs were collected from Total 6 houses and six poultry farms. Hens were tagged and six eggs were collected from one hen on consecutive days and then they were used for further analysis. **Control samples of chicken eggs were also collected for comparison from organic farm**. Samples were kept in ice box during their transportation to the testing laboratory where they were kept at 4 °C until analysis. They were analyzed for presence of pesticide residues within 24 h from their arrival.

2.2 Selection of pesticides

Primary data was collected from study area. On the basis of field survey and interviews with farmers, poultry farm owners and residents about the types, amount and frequency of pesticides that are sprayed during surrounding agricultural activities; pesticides/insecticides selected for the research include Difenoconazole, Imidacloprid and Bifenthrin.

2.3 Sample extraction and clean up

Approximately 6 eggs (20-30 gm) of each hen were taken in a beaker and mixed thoroughly followed by the addition of 30g anhydrous sodium sulphate for water removal. This mixture was placed on a magnetic stirrer for 15 minutes for homogenous mixing followed by Soxhlet extraction with 100ml of petroleum ether each time (this process was repeated five times) followed by extraction with acetonitrile: petroleum ether in a ratio of (50:50). Organic layer was collected and was kept under nitrogen atmosphere for 20 min followed by the addition of anhydrous sodium sulphate (20 gm). This mixture was then filtered and concentrated on rotary evaporator to a volume about 3ml it was again dried over anhydrous sodium sulfate and was filtered via mirofilter before storation in a vail for further HPLC analysis. Pesticide standards were diluted with methanol and filtered via micro filters before there HPLC analysis (Ahmad et al., 2010). Pesticide residues were detected and determined according to the Association of Official Analytical Chemists (1995) methods.

2.4 Analytical analysis

HPLC conditions, method development and quality control

After extraction and solvent evaporation, the samples were analyzed according to the proposed method. For analysis, HPLC (Agilent 1260, Quaternary gradient system) was used under the following conditions. A mobile phase was acetonitrile and water (HPLC Grade) in ratio of 40:6 0. Both of them were filtered through cellulose filters (0.2 µm). All other parameters were optimized for Chlorination byproducts. HPLC column Zorbax-Eclipse plus C 18, 4.6 × 1000 mm, 3,5, microns the run was 2 hr, flow rate optimized as 1 ml/min, column temperature was kept at 25 °C, injection volume maintained at 5 µL and UV detection at 245 nm. pH was adjusted to 4.5 with phosphoric acid. All analytes were subjected to stringent quality control methods. Before sample analysis, the instruments were calibrated with calibration standards. The target analytes were identified and quantified by comparing the retention times and peak area of the sample with those of the calibrated internal standards (reference standards).

All analyses were carried out in triplicate and the mean concentrations were considered for precision and accuracy. Blank analyses were also performed in order to check interference from the sample. Reproducibility of sample was satisfactory. A gradient rapid and sensitive HPLC-UV method was developed and validated to determine pesticides in chicken eggs. To achieve method development optimization studies were performed on each HPLC parameter such as solvent ratio, pH, temperature of column, sample and injection volume, flow rate, wavelength and post time etc. For optimization, one parameter was changed at one time while all others were kept constant. Calibration experiments were tested for linearity, accuracy and precision. Limit of detection (3:1) and limit of quantification (10:1) was calculated as signal-noise ratio and they were found to be 0.01 and 0.03.

2.5 Quantitative analysis

Qualitative analysis was carried out to check the presence of selected pesticides in the samples on the basis of retention times. For quantitative analysis Equation 1 and Equation 2 were used (LC/GC Chrom Academy, 2014).

$$\text{Response factor} = \frac{\text{Peak Area of standard}}{\text{Standard Amount}\left(\text{used in1ml of solvent}\right)} \quad (1)$$

$$\text{Amount of standard in sample} = \frac{\text{Peak Area}\left(\text{sample peak}\right)}{\text{Response Factor of detected pesticides}} \quad (2)$$

2.6 Assessment of health risk

The entire population was considered potentially exposed, as everyone is approximately utilizing eggs. Health risk estimates for pesticide residues in chicken eggs was computed using two basic standard indices: the Estimated Average Daily Intake (EADI) and the acceptable daily intake (ADI). Degree of risk associated within the consumption of each pesticide detected (present in chicken eggs) was monitored by evaluating the results of pesticides residues detected in samples. **Health risk index (HRI)** was calculated by using estimated daily intake (EDI) and acceptable daily intake (ADI). The value of acceptable daily intake for bifenthrin and difenoconazole is **0.01 ppm.** The EDI was calculated by multiplying average consumption of a person per day (kg/day) and residual concentration of pesticide (mg/kg) and dividing by average weight of an Asian (60 kg) (Abubakar et al., 2015; Hossain et al., 2013; Balkhair & Ashraf, 2016). The average consumption of chicken eggs was considered 0.0425 person/day (approximate weight of one egg). Health risk to consumers was assessed by calculating health risk index using formulas as given in Equation 3 and Equation 4 (Mahmood & Malik, 2014)

$$\text{Estimated Daily Intake} = \frac{\text{Residual Pesticide concentration} \times \text{Food consumption rate}\left(\text{kg / day}\right)}{\text{Body Weight for an adult}\left(60\ \text{Kg}\right)} \quad (3)$$

$$\text{Health risk index} = \frac{\text{Estimated Daily Intake}}{\text{Acceptable Daily Intake}} \quad (4)$$

3 Results and discussion

Eggs contain a high fat percentage and will accumulate persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), dioxins and pesticides. Thus the purpose of the presented study was to assess the health risk to human beings due to utilization of chicken eggs contaminated with different pesticides.

For this purpose first, the presence of pesticides was assessed and quantified by HPLC analysis. A gradient and sensitive HPLC method using water and acetonitrile solvent system was successfully employed. No interfering peaks for the determination of pesticides were observed. The presence of **Organochlorine pesticide** residues in consumable chicken eggs poses health risks to general public and is reported by many studies before. Pesticides are transferred to chicken feed which contaminates the chicken eggs and meat. The contaminated food when consumed by human beings also badly affects them. Results revealed the presence of pesticides i.e. Bifenthrin and difenoconazole in all egg samples with different concentrations. While imidacloprid was not detected in any of the egg sample. However no pesticide was detected in controlled samples. Bifenthrin is an effective pesticide which is used in surrounding agricultural fields for vegetables, fruits and cotton pest control. It is also intended for public health to control mosquitoes. Bifenthrin was detected in all samples in concentration of 1.5862 mg/kg, 1.78792 mg/kg, 4.257713 mg/kg, 2.698864 mg/kg, 5.80796 mg/kg, and 2.78905 mg/kg for poultry farm samples while in 2.79347 mg/kg 4.112387 mg/kg, 0.256206 mg/kg, 0.53951 mg/kg, 3.1748 mg/kg, and 3.18781 mg/kg concentration in egg samples collected from houses. Difenoconazole is mainly a fungicide and pesticide as well was found in concentration 10.939 mg/kg 12.3296 mg/kg, 29.3617mg/kg, 18.6116 mg/kg, 40.0523 mg/kg, 19.2335 mg/kg in poultry farm samples while in house samples the detected concentration was 0.02835 mg/kg, 1.7668 mg/kg, 3.7205 mg/kg, 21.8937 mg/kg, 21.9835 mg/kg and 19.26407 mg/kg (Table 1). Commissions of the Food and Agriculture Organizations (FAO) UN have established MRLs to avoid the human exposure to pesticide through food consumption (Food and Agricultural Program of the United Nation, 1997).

The maximum residual limits for both of the detected pesticides are 0.05mg/kg and results revealed that in approximately all collected samples the detected concentration of both pesticides was exceeding the MRLs thus posing potential risk towards the health of consumers. Not only the detected levels very high, but some of the egg samples were containing multiresidues of both types of detected pesticides. Exposure to pesticides through contaminated egg consumption to human beings leads to a spectrum of adverse health effects that depend on the nature of the pesticide and on the amount and duration of exposure. Difenoconazole contamination in food with the values above the MRLs may result in human toxicity including carcinogenicity, neurotoxicity, reproductive and developmental toxicity and acute toxicity as indicated by previous studies (European Food Safety Authority, 2011a). Bifenthrin in food may cause allergic reactions, dermatitis, asthma, bronchitis, nasal stuffiness and sneezing (European Food Safety Authority, 2011b). Extensive use and low biodegradability of pesticides may also contaminate the soil and other environmental sources. As the mean detected pesticide residues concentration in the chicken eggs was exceeding the MRLs, thus the persistent and bio-accumulative nature of pesticides is of great concern because of the possible build up to toxic levels. Different studies also supported the presence of different organochlorinated pesticides (OCP) in poultry feed, chicken muscles and eggs in mean concentration of 0.65, 0.91, 0.42 and 0.02 mg kg^{-1} etc which are less than the values reported in our research (Rabinder et al, 2016). Results of another on Organochlorine pesticide residues in eggs, chicken and mean in Jordan revealed that 28% (38/134), 20% (23/115) and 49% (131/270) of the examined eggs, chicken and meat samples, respectively, were contaminated with OCP residues (Ahmad et al., 2010). Contamination of these pesticides is also well reported (Hossain et al., 2013) in other food items like vegetables, fruits etc as for example results of a study conducted for the determination of difenaconazole and bifenthrin revealed the presence of high level of their contaminants in different fruit samples in difenaconazole concentration of 209 ppm and 333ppm and bifenthrin in concentration of 132 ppm and 210 ppm. The detected concentration of these pesticides was higher those detected in egg values (Khan et al., 2015).

The level of the risk imparted towards health due to these pesticides present was further assessed by calculating the

Table 1. Detected Concentration of Pesticides and calculated HRI in Chicken egg samples.

Sr. No	Sample	Difenoconazole Concentration mg/kg	HRI 60	Health Risk	Bifenthrin Concentration mg/kg	HRI 60	Health Risk
1	Poultry farm 1	10.939	0.7748	No	1.5862	0.00112	No
2	House 1	0.02835	0.002	No	4.112387	0.29129	No
3	Poultry farm 2	12.3296	0.8733	No	1.78792	0.1267	No
4	House 2	1.7668	0.125	No	0.256206	0.0018	No
5	Poultry farm 3	29.3617	2.0797	Yes	4.257713	0.3015	No
6	House 3	3.72051	0.2635	No	0.53951	0.0379	No
7	Poultry farm 4	18.6116	1.3183	Yes	2.698864	0.00191	No
8	House 4	21.8937	1.5508	Yes	3.1748	0.2248	No
9	Poultry farm 5	40.0523	2.83703	Yes	5.80796	0.4113	No
10	House 5	21.9835	1.5571	Yes	3.18781	0.2258	No
11	Poultry farm 6	19.2335	1.3623	Yes	2.78905	0.1975	No
12	House 6	19.26407	1.3645	Yes	2.79347	0.1978	No
13	Control samples	ND	---	---	ND	---	---
	MRL / Cutoff value for HRI	**0.05**	**1**		**0.05**	**1**	

ND = not detected.

health risk index. Health risk calculations (Carmen et al., 2008) showed that health risk index exceeded 1 (the cut off value) for Difenoconazole in seven samples while for Bifenthrin its value did not exceed 1 for any sample (Table 1). Results indicate that there is the possibility of potential health risk associated with exposure to detected pesticides through eggs to human beings utilizing them especially because of presence of difenoconazole as HRI for difenoconazole was the highest in most of samples. Regular monitoring of the use of common pesticides especially in developing countries should also be undertaken for regular monitoring of residual pesticide levels that may pose potential health hazard. **Adaptation of best agricultural practices in the surrounding area and best management practices in the poultry farms may help to avoid or reduces the chances of contamination.**

4 Conclusion

The present study has shown the accumulation of pesticide residues i.e., Bifenthrin and Difenoconazole in chicken egg samples in concentration exceeding the MRLs however pesticide Imidacloprid was found to be absent in all samples. Health risk calculations showed that health risk index exceeded 1 (the cut off value) for Difenoconazole in seven samples while for Bifenthrin its value did not exceed 1 for any sample. Results indicate that there is the possibility of potential health risk associated with exposure to detected pesticides through eggs to human beings utilizing them. **As the poultry feed is the major source of pesticides in chicken eggs thus poultry feed requires quality control and monitoring and their breeding should also be in confined area following best management practices of poultry farms.**

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