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A Study of Pesticide Residues in Different Fruits Collected from Different Fruit Markets of Lahore, Punjab

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Abstract

Pesticides are poisons in nature and have harmful effects on human health. They are used for suicide, homicide and for other bad intentions. ***Objective:*** The aim of the study was to determine the residues of some pesticides in fruits collected from various markets of Lahore. ***Material & Methods****:* Eight fruits samples apple, banana, guava, melon, orange, papaya, pomegranate and strawberry from various sale points of the market were collected. Extraction, clean up and analysis were then processed. Concentration of five pesticides including Bifenthrin, Lambda Cyhalothrin, Carbofuran, Chlorpyrifos and Cypermethrin were determined in fruits samples using high performance thin layer chromatography. Spot diameter of pesticide residue of sample was compared with that of standards to determine pesticide concentration. One way ANOVA was applied to determine the significance of difference. ***Results & Cnoclusion:*** Significant difference of pesticide presence was identified in some selected fruits ($P<0.05$). The maximum concentration of cyhalothrin was found in Papaya (0.112 mg/kg) and Strawberry (0.133mg/kg), Cypermethrin in Orang (0.320 mg/kg), Banana (0.170 mg/kg) and Pomegranate (0.129 mg/kg), Chlorpyrifos in Guava (0.122 mg/kg), Carbofuran in Apple (0.109 mg/kg). It was concluded that cypermethrin possess high rate of contamination in different fruit samples. Therefore, there is a need to continuity of inspection on pesticide residues in agricultural products in order to prevent the contamination and secure human safety.

Keywords: pesticide, hygienic, cypermethrin, carbofuran, ANOVA

1. Introduction

Pakistan is one of the agricultural countries. Agricultural sector is the backbone of economy. Exports of these agricultural products such as fruits are an important source of making foreign exchange in order to boost exports of Pakistan [1]. Fruits contain the essential elements of human diet. Fruits are used to accomplish the requirements of balanced diet. Fruits contain different nutritional elements such as different vitamins, minerals and antioxidants. Antioxidants play an important role in the metabolic pathways and inhibit the abnormal growth of cells [2]. Pesticides are used to increase the yield of agricultural products. Pesticides contain different disreputable elements that kill pests. These elements are not specific for pests but also have harmful effects on human beings [3]. Different techniques can be used to apply pesticides. As the use of pesticides increases, the contact of human to these poisonous elements also increases [4].

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The application of different pesticides results in the contamination of soil, water and food. These pesticides not only stick to the crops and fruits but also absorb inside of the fruits that can transfer to human when they consume them. These pesticide residues are dangerous for environment and human health too. Different classes of pesticides are used on the basis of their active ingredients. Pesticides reside in the food elements on which these are applied. Ingestion of these noxious elements is called toxicity. Maximum residue limit of each pesticide is different. Different lab animals are used to check the maximum residue limit. These elements can reside in human fat or breast milk [5]. Pesticides cause hostile effects on human health. Many synthetic pesticides cause hormonal imbalance in human [6]. Pesticides basically act on the neurotransmitters; this is not specific for pests. Pesticides residues can cause acute or chronic diseases. Different types of pesticides are used in Pakistan to control pests and increase the crop yield. The increased use of pesticides results in the contamination of food and environment. These pesticides act on human through dermal, oral and inhalation and cause deleterious effects.

2. Materials and methods

Residues of different pesticides were tested in eight various fruits using High Performance Thin Layer Chromatography (HP-TLC).

2.1. Pesticides selection. Those pesticides were selected which are commonly used in Pakistan given in (Table 1).

2.2. Collection of samples.The samples of apple, orange, banana, melon, pomegranate, papaya, guava and strawberry were collected from various sale points of different fruit markets of Lahore. Samples were taken randomly at different times and placed in polythene bags and put in refrigerators at 4^{o}C to avoid degradation of pesticide residues. Some residues become difficult to identify due to the presence of trace amount that can never be determined by high performance thin layer chromatography.

Common and scientific names of selected fruits are given in (Table 2).

2.3. Extraction methodology. The collected fruits were preserved at -4˚C. The preserved samples were taken out from refrigerator one an hour before further procedure. These samples thawed and chopped in a high speed blender. In Erlenmeyer flask of 500ml, 20 g from chopped fruit sample and 20 g of anhydrous sodium sulfate, 2.5 g of NaCl and 70 ml ethyl acetate was added. Extraction of pesticides can be done in acetone, methanol or ethyl acetate. But ethyl acetate was easily available so it was used for extraction. Flask was placed on an electric shaker for one an hour. Aqueous material was passed through Whattman filter paper No.42 for separation from other solid materials (not soluble in ethyl acetate) [4].

2.4. Clean-up procedure. Clean up procedure was performed to clean the fruit extract. Pesticide residues were soluble in ethyl acetate so these residues can pass through different cleaning steps: Extracted sample was cleaned up by passing it through activated charcoal. Charcoal was activated at 105˚C for 4 hours. The extract was put in round bottom flask. Residues were dissolved in acetone [7]. Sample was concentrated by rotatory evaporator to make residue concentrated to identify. Samples were filtered through 0.2 μm filter paper and analyzed using HP-TLC [4].

2.5. Preparation of standards. Standards were available in different percentage and in powder form. 1% standards solution was prepared in methanol (Table 3).

2.6. High Performance Thin Layer Chromatography (HPTLC). High performance thin layer chromatography was used for the detection and calculation of concentration of pesticides.

2.6.1. Methodology. Glass plates were prepared with silica gel having 0.25 mm thickness. Plate was activated at 105°C for 30 minutes. First of all spotted the different concentrations of standards, noted the concentration and diameter of spot after the development of plate. A graph was drawn between concentration and spot diameter to assess concentration of pesticides in samples. The extracted sample was spotted along with the standards. Plate was placed in tank that contained methanol and ammonia as a mobile phase. Plate was placed in fume hood to remove the excess material.

2.6.2. Development of plate. All pesticides were not detected through same detection procedure; different techniques were used to develop the plate. Some

pesticides provided fluorescence when observed under UV at 254 nm for 30 minutes. But many pesticides did not give fluorescence under UV light therefore different coloring techniques were applied to develop plate.

2.6.3. Potassium iodide and O-tolidine: Spray of potassium iodide and O-tolidine give brown yellowish color. Coloring reagent spray was chosen to note down the diameter of spots. Color intensity varies with respect to the concentration of pesticides in standards and sample spots. Therefore, it was useful in determining the concentration of pesticide residues.

2.6.4. Enzyme inhibition coloring reagents: This coloring technique was used for the development of carbofuran on HPTLC plate. The preparation methodology which was followed is given below.

2.6.5. Preparation of reagent solution: Sodium 2, 6-Dichlorophenolindophenol was used as reagent solution for the development of high performance thin layer chromatography. Chemical formula of Sodium 2, 6- Dichlorophenolindophenol is $C_{12}H_6C_{12}NNaO_2 \cdot nH_2O$. It is a redox chemical compound; when oxidizes it produces blue color and when reduces, produces colorless compound.

2.6.6. Enzyme solution preparation: Acetyl cholinesterase enzyme was used for the development of high performance thin layer chromatography.

Enzyme Inhibition Method. This technique was used for the development of those pesticides which did not fluoresce under UV light on plate and produce color by potassium iodide and O-tolidine. Plate was placed in bromine vapors for 15 minutes. Excess vapors were removed from plate by placing in fuming hood for 45 minutes. Horse blood serum enzyme was sprayed on the plate and placed at 37°C for 30 minutes. Then substrate solution was sprayed to develop color. Spray was carried out in incubator. Superfluous vapors were removed. Then the reagent solution was sprayed. Blue spots appear against the white background. Blue spots indicate the presence of carbamate pesticides. The distance was measured which travelled by the ethyl acetate and the pesticides spots. The area of spots both horizontally and vertically was measured to calculate concentration. The R_f value and concentration was measured through this procedure [7].

2.7. Identification of pesticides. After developing the plate it was observed that which pesticide was present in extracted fruit sample. If retention factor of samples pesticide was in agreement with the standard retention factor, it was determined that the sample was contaminated with pesticide.

2.8. Quantitation of pesticide. Quantitation of pesticides from high performance thin layer chromatography was done by measuring the spot diameter of standards for different concentrations. Then perform analysis for different samples and develop the plate. Measure the spot diameter and compare with standard spot diameter then it will be clear how much concentration of pesticide is present in sample.

3.Results

3.1. Detection techniques. Different fluorescent and coloring techniques were applied to develop the spots. Almost all pesticides with some exceptions gave fluorescence under UV light. But for measuring diameter, coloring technique has to be applied. All pesticides were not colored by the same coloring reagent. Different coloring reagents produce different colors (Table 4).

3.2. Retention factor. Retention factor of each pesticide was calculated by running the standard samples on HPTLC plate. Retention factor was calculated by measuring the distance travelled by the spots and solvent.

R_f =Distance travelled by the spot/ distance travelled by the solvent.

Retention factors of used pesticides are given in (Table 5).

3.3. Development of standard concentration. Before analyzing the samples, different concentrations of standards were spotted on plate and developed the graph between concentration verses developed spot diameter. This graph is considered as standard. The prepared standard solution was 1% and contained the calculated amount. From that calculated amount it was drawn that 2 micro liters contain how much concentration of pesticide. The spot diameter indicates that how much concentration of standard can produce a spot of this diameter. When calculated amount of

sample was spotted, the resulted diameter of spot was compared with standard diameter and calculated the concentration of pesticide. Concentration of pesticides. The calculated concentration of pesticides in different fruits is given in (Table 6).

Table 1. Selected pesticides

Pesticides name	Molecular formula	Classification
Lambda cyhalothrin	$C_{23}H_{19}ClF_3NO_3$	Pyrethroid
Cypermethrin	$C_{22}H_{19}C_{l2}NO_3$	Pyrethroid
Chlorpyrifos	$C_9H_{11}C_{l3}NO_3PS$	Organophosphate
Bifenthrin	$C_{23}H_{22}ClF_3O_2$	Pyrethroid
Carbofuran	$C_{12}H_{15}NO_3A$	Carbamate

Table 2. Selected fruits

Common name	Scientific name
Apple	Malus domestica
Banana	Musa sapientum
Guava	Psidium guajava
Orange	Citrus aurantium
Pomegranate	Punica granatum
Papaya	Carica papaya
Strawberry	Fragaria

Table 3. Standards preparation

Standard % available	Quantity of standard taken (g)	Total volume (solvent)
Bifenthrin (95.03%)	0.105	10 ml
Chlorpyrifos (97.03%)	0.103	10 ml
Cypermethrin (97.03%)	0.103	10 ml
Carbofuran (92%)	0.108	10 ml
L-Cyhalothrin (95.03%)	0.015	10 ml

Table 4. Detection of pesticides

Pesticides	Detection on HPTLC
Lambda Cyhalothrin	UV light at 254 nm and o-Tolodine and potassium iodide
Carbofuran	UV light at 254 nm and enzyme inhibition with horse blood serum and acetylthiocholine iodide substrate (EAcl)
Cypermethrin	UV light at 254 nm and o-Tolodine and potassium iodide
Chlorpyrifos	UV light at 254 nm and o-Tolodine and potassium iodide
Bifenthrin	UV light at 254 nm and o-Tolodine and potassium iodide

Table 5. Retention factor for pesticides

Pesticide name	Distance travelled By spot (cm)	Distance travelled by solvent (cm)	Retention factor
Cypermethrin	8.5	15	0.566
Chlorpyrifos	8.9	15	0.593
Bifenthrin	6.8	15	0.453
Lambda cyhalothrin	6.9	15	0.460
Carbofuran	8.5	15	0.566

Table 6: Pesticides Concentration

	Pesticide concentration in mg/kg				
Fruits	**Bifenthrin**	**Carbofuran**	**λ-Cyhalothrin**	**Cypermethrin**	**Chlorpyrifos**
Apple	0.076	0.109	0.024	0.108	0.072
Banana	0.09	0.084	0.120	0.170	0.060
Guava	0.06	0.047	0.049	0.070	0.122
Melon	0.058	0.067	0.041	0.069	0.061
Orange	0.107	0.070	0.118	0.32	0.132
Papaya	0.114	0.057	0.133	0.094	0.065
Pomegranate	0.105	0.052	0.045	0.129	0.035
Strawberry	0.112	0.059	0.112	0.035	0.026

3.4. Statistical analysis. Analysis of variance (ANOVA) test was applied and concluded that both in fruits and pesticides, significant difference is present ($P<0.05$). By applying least significant difference test it was concluded that cypermithrin was present in large concentration as compared to the other pesticides. All the fruits were found contaminated with pesticides irrespective of market. If fruits are used after proper peeling then the risk of pesticides infestation will be minimum.

4. Discussion

Different kinds of pesticides are used to avoid the pest infestation to the fruits. If intended maximum residue limit of pesticides exceeds, then causes different kinds of diseases Khan et al. (2011) [4]. UV light gave florescence to chlorpyrifos and lambda cyhalothrin, the spots became visible same as determined by Manzoor et al. (2012) [8]. Detection of different classes of pesticides such as organophosphate, pyrethroid and carbamates was determined by UV light as studied by Ambrus et al. (2002) [9]. Concentration to develop carbamate by enzyme inhibition method was examined by Asi et al. (2003) [10]. It was determined that retention factor of pesticides has no environmental factors. Retention factor of pesticides were determined same as Liu and Qian. (2002)[11]. Retention factor was obtained by measuring the distance travelled by the spot and the distance travelled by the solvent. Retention factor of these pesticides are in accordance with the results of Asi et al. (2003) [10]. Retention factor calculated from HPTLC for cypermitherin and chlorpyrifos had resemblance with the results of Manzoor et al. (2012) [12]. HPTLC was used for the determination of pesticides in fruits. This technique is also used by the Asi et al. (2003) [10] and Iqbal et al. (2009) [7]. Lambda cyhalothrin is a pyrethroid pesticide, used as an insecticide. It was determined that strawberry, orange and papaya were contaminated with cypermethrin. Significant difference is present in concentration of outer portion and inner portion of fruits. Bifenthrin residues were mostly determined in strawberry and in accordance with Asi et al. (2003) [10]. Carbofuran is a carbamate pesticide. When it sprayed in field then it becomes the part of fruit and no pests can destroy the fruits Fernandez et al. (2000) [8]. Chlorpyrifos belongs to organophosphate class of pesticides. Significant difference is present in absorption of cypermethrin as compared to other pesticides; these results were also discussed by Asi et al. (2003) [10]. Quantitation of pesticides in this study was done by measuring the diameter of standards spots along with different concentrations.

5. Conclusion

Since all the fruits which were analyzed are contaminated with pesticide residues. No doubt, pesticides degraded within days but the degraded elements remain in fruits. Regular checking of fruits collected from different markets should be done with some more advanced and sophisticated instruments to monitor all other agricultural commodities establish regional guidelines for the post-harvest period. There is a need to continue this work and examine all other agricultural products including milk, meet and other foods to secure the human safety**.**

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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 / or animal subjects (if exist) respect the specific regulation and standards.

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