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Identification of pesticide residues in different vegetables Collected from market of Lahore, Pakistan

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Abstract

This research work was done in Punjab forensic science agency in 2013 and it was concluded that regular checking of vegetables should be done to maintain the hygienic level of vegetables. Concentration of six different pesticides Imidacloprid, Chlorpyrifos, Cypermethrin, Lambda cyhalothrin, Carbofuran and Bifenthrin belonging to different classes were determined in fifteen different local vegetable samples by using high performance thin layer chromatography. Diameter comparison was done between standard and sample spot to determine the concentration. Significant difference was detected in presence of pesticides in various vegetables ($P<0.05$).The highest concentration of pesticides was; Imidacloprid in Okra (0.131 mg/kg), Bifenthrin and Chlorpyrifos in Potato (0.109 mg/kg) and (0.124 mg/kg) respectively, Carbofuranin in Tomato (0.120 mg/kg), Lambda cyhalothrinin in Cucumber (0.114 mg/kg), Cypermethrin in Egg Plant (0.090 mg/kg). It was concluded that cypermethrin and carbofuran possess high rate of contamination of different vegetables samples.

Keywords: Imidacloprid, Chlorpyrifos, Cypermethrin, Lambda cyhalothrin, Carbofuran, Bifenthrin, HPTLC, Maximum Residue Limit

1. Introduction

Pakistan is one from agricultural countries. Agricultural sector is an important sector in accordance with economy. Exportation of these agricultural products such as vegetables is the main source to improve the economy of Pakistan [10]. Vegetables contain the essential elements of human diet. Vegetables are frequently used in different areas to fulfill the balanced diet requirements. Vegetables contain different nutritional elements such as different types of vitamins, minerals and antioxidants.

Antioxidants play important role in the metabolic pathways and nullify the tumor cells [11]. Pesticides are used to increase the yield of agricultural products. Pesticides contain different notorious elements that kill pests. These elements are not specific for pests, can also act on human beings [12]. Different means of applications can be used to apply pesticides or insecticides and act on human beings directly or indirectly. As the use of pesticides increases in fields the contact of human beings to these poisonous elements also increases [6]. The application of different pesticides on field; results in the contamination of soil, water as well as food elements.

These pesticides attach with the crops and vegetables but also absorb in the deep of the vegetables that can

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transfer to human beings when they consume these items. These pesticide residues are dangerous for our environment and human health. Different classes of pesticides are present on the basis of active ingredients. Pesticides can reside in the food elements on which pesticides spray was used. Intake of these poisonous elements is called toxicity. Maximum residue limit of each pesticide is different and calculated before it came in field. Different lab animals use to check the maximum residue limit. Also calculate the dose to time which means that at which concentration this pesticide can cause disease to human being. These elements can reside in human fat or breast milk [8]. Pesticides cause unfavorable effects on human health. Many synthetic pesticides cause hormonal imbalance in human beings [9]. Pesticides basically act on the neurotransmitter; this property is not specific for pests. Pesticides residues can cause acute or chronic infection. Different sprays of pesticides are used in Pakistan to control pests and to increase the yield of crops. The increased use of pesticides results in the contamination of food and environment. Through dermal, oral and inhalation these pesticides act human beings and cause long term or short term diseases. The present study was designed to check the contamination of different pesticides in local vegetables.

2. Materials and Methods

Residues of different pesticides were checked in fifteen different vegetables by using the 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).

Table 1. Selected pesticides

Pesticides name	Molecular formula	Classification
Lambda cyhalothrin	$C_{23}H_{19}ClF_3NO_3$	Pyrethroid
Cypermethrin	$C_{22}H_{19}Cl_2NO_3$	Pyrethroid
Chlorpyrifos	$C_9H_{11}C_{l3}NO_3PS$	Organophosphate
Bifenthrin	$C_{23}H_{22}ClF_3O_2$	Pyrethroid
Imidacloprid	C9H10ClN5O2	Insecticide (neonicotinoid)
Carbofuran	$C_{12}H_{15}NO_3A$	Carbamate
Endosulfan	$C_9H_6C_{l6}O_3S$	Organochlorine

2.2.Collection of samples. All vegetables were collected from different vegetable markets of Lahore. Samples were transferred in polythene bags, and then transferred to refrigerators to avoid changing because pesticides degrade rapidly into its residues. Some residues become difficult to identify, may be due to the presence of minute quantity that can never be determined by high performance thin layer chromatography.

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

Table 2. Selected vegetables

Common name	Scientific name
Pumpkin	*Curcurbita maxima*
Carrot	*Daucuscarota*
Potato	*Solanumtuberosum*
Tomato	*Solanumlycopersicum*
Cucumber	*Cucumissativus*
Egg plant	*Solanummelongena*
Cabbage	*Brassica oleracea*
Radish	*Raphanussativus*
Okra	*Hibiscus esculenta*
Bitter gourd	*Momordicachorantia*
Spinach	*Spineraciaoleracea*
Cauliflower	*Brassica oleracea*
Squash	*Cucurbitapepo*
Turnip	*Brassicarapa*
bell pepper	*Capsicum annuum*

2.3. Extraction methodology. Fresh vegetables were collected from the vegetable markets of Lahore.

The collected vegetables were preserved at -4 ˚C. The preserved samples were taken off after passing one an hour for further procedure. Thawed and chopped these samples with speed blender. 500 ml Erlenmeyer flask was selected, added in it100 g from chopped vegetable sample. 20 g of anhydrous sodium sulfate, 2.5 g of NaCl and 70 ml freshly prepared distilled 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. Flasks were shacked for one an hour. Aqueous material was passed through Whattman fluted filter paper No.42 for separation from other solid materials that were the vegetable parts (not soluble in ethyl acetate) [4].

2.4. Clean-up procedure. Clean up procedure was performed to clean the extract of vegetables.

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 shifted in round bottom flask (1 ml) [4]. Residues were dissolved in acetone [4]. Sample was concentrated by rotatory evaporator when sample was passed through this step then it was considered that pesticide residues become concentrated and easy to identify [6]. Samples were filtered through 0.2 µm filter paper and analyzed by HP-TLC [6].

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

Table 3. Standards preparation

Standard % available	Amount taken of standard (g)	Total volume (solvent)
Imidacloprid (95.03%)	0.105	10 ml
Bifenthrin (97.03%)	0.103	10 ml
Chlorpyrifos (97%)	0.103	10 ml
Cypermethrin (92%)	0.108	10 ml
Carbofuran (97%)	0.103	10 ml
Lambda cyhalothrin (96%)	0.104	10 l

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

2.6.1. Methodology. Glass plates were prepared of 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 that was helpful in the assessment 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 for the purpose to remove the extra material.

2.6.2. Development of plate. All pesticides were not detected through same detection procedure; different techniques were followed for the development of plate. Some pesticides gave fluorescence when observed under UV at 254 nm for 30 minutes. But many pesticides were not giving flourishing under UV light, then different coloring techniques were also applied to develop plate.

2.6.3. Potassium iodide and O-tolidine. Spray of potassium iodide and O-tolidine produce brown yellowish color. Coloring reagent spray was preferred to note down the diameter of spots. Color intensity of this color varies with respect to the concentration of pesticides in standards and sample spots. That’s why it was helpful in determination of concentration.

2.6.4. Enzyme inhibition coloring reagents. This coloring technique was used for the development of carbofuran on HPTLC plate. The preparation methodology that 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 (HPTLC). 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 this compound oxidizes then producesblue color and when reduces then 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 give florescence under UV light on plate and produce color by potassium iodide and O-tolidine.

Plate was placed in bromine vapors for 15 minutes.Exceeding vapors were removed from plate by placing it again in fuming hood for 45 minutes.Horse blood serum enzyme was sprayed on the plate for 30 minutes at 37°C. Hot air steam was given to remove the extra vapors from plate. Then substrate solution was sprayed to develop color. Spray was done in incubator. Extra vapors were removed. Then the reagent solution was sprayed.

Blue spots appear against the white background. Blue spots indicate the presence of carbamatepesticides.

The distance was measured, covered by the ethyl acetate and the pesticides spots. The area of spots both horizontally and vertically was measured to calculate concentration. Calculated the R_f value and concentration was measured through this procedure [4].

2.7. Identification of pesticides

After developing the plate it was observed that which pesticide was present in vegetable extracted sample. If retention factor of samples pesticide was matched with the standard retention factor it was concluded 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 diameter of standards for different concentrations. Than run different samples and develop the plate. Measure the diameter and compare with the standard diameter from this it will be clear that 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 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.

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

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

Table 4. Detection of pesticides

Pesticides	Detection on HPTLC
Imidacloprid	UV light at 254 nm and o-Tolidine and potassium iodide
Lambda cyhalothrin	UV light at 254 nm and o-Tolidine and potassium iodide
Carbofuran	UV light at 254 nm and Enzyme inhibition with horse blood serum and acetylthiocholine iodide substrate (EAcI)
Bifenthrin	UV light at 254 nm and o-Tolidine and potassium iodide
Cypermethrin	UV light at 254 nm and o-Tolidine and potassium iodide
Chlorpyrifos	UV light at 254 nm and o-Tolidine and potassium iodide

Table 5. Retention factor for pesticides

Pesticide name	Distance travelled by spot (cm)	Distance travelled by the solvent (cm)	Retention factor
Cypermethrin	8.8	13	0.67
Chlorpyrifos	8.7	13	0.669
Bifenthrin	6.8	13	0.52
Lambda cyhalothrin	7	13	0.540
Carbofuran	8.2	13	0.630
Imidacloprid	3	13	0.23

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 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 vegetables is given in (Table 6).

Table 6. Pesticides concentration

Vegetables	Pesticides concentration in mg/kg					
	Imidacloprid	Bifenthrin	Carbofuran	Lambda cyhalothrin	Cypermethrin	Chlorpyrifos
Pumpkin	0.063	0.063	0.078	0.088	0.050	0.074
Okra	0.131	0.065	0.103	0.067	0.062	0.088
Egg plant	0.108	0.054	0.059	0.057	0.096	0.114
Bitter gourd	0.030	0.037	0.108	0.061	0.076	0.053
Potato	0.108	0.109	0.110	0.108	0.083	0.124
Tomato	0.091	0.105	0.120	0.096	0.018	0.062
Radish	0.039	0.047	0.089	0.059	0.026	0.034
Carrot	0.056	0.061	0.034	0.092	0.023	0.038
Bell pepper	0.084	0.075	0.063	0.106	0.084	0.075
Turnip	0.044	0.065	0.079	0.056	0.046	0.037
Squash	0.065	0.055	0.073	0.071	0.063	0.117
Spinach	0.080	0.103	0.095	0.052	0.087	0.086
Cucumber	0.072	0.084	0.108	0.114	0.084	0.099
Cabbage	0.052	0.068	0.062	0.063	0.051	0.036
Cauliflower	0.079	0.049	0.109	0.084	0.051	0.057

3.4.Statistical analysis. Ftest was applied and it was concluded that ($P<0.05$) in both vegetables and pesticides significant difference is present.

By applying least significant difference test it was concluded that cypermethrin was present in large concentration as compared to the other pesticides. Mostly vegetables were contaminated, but mostly residues were present in leafy portion of vegetables. If vegetables will be used after proper peeling then the risk of pesticides infection will be reduced.

4.Discussion

Different kinds of pesticides can be used to avoid the contamination of vegetables. If calculated maximum residue limit of pesticides exceeds, then cause different kinds of diseases Khan et al. (2011) [6]. UV light gave florescence to chlorpyrifosand imidacloprid, the spots became visible same as determined by Manzoor et al. (2012) [5]. Detection of different classes of pesticides such as organophosphate, pyrethroid and carbamateswas determined by UV light as studied by Ambrus et al. (2002) [1]. Results of development of carbamate by enzyme inhibition method were examined by Asi et al. (2003) [2]. It was determined that retention factor value of pesticides can never be changed by any environmental factor or regardless of any time. Retention factor of pesticides were determined same as Liu and Qian. (2002) [7]. Retention is calculated by measuring the distance travelled by the spot and the distance travelled by the solvent. Retention factor of these studied pesticides exactly matched with the results of Asi et al. (2003) [2]. Retention factor calculated from HPTLC for imidacloprid and chlorpyrifos had resemblance with the results of Manzoor et al. (2012) [5]. HPTLC was used for the determination of pesticides in vegetables. HPTLC can be used for detection of different kinds of pesticides by applying different coloring reagents or under UV light. This technique is also used by the Asi et al. (2003) [2] and Iqbal et al. (2009) [4]. Lambda cyhalothrin is a pyrethroid pesticide, used as an insecticide. Residues of lambda cyhalothrin were mostly determined in pumpkin. Pumpkin contained high absorption rate of pesticide Asi et al. (2003) [2]. It was determined that cucumber, cabbage and cauliflower were contaminated with lambda cyhalothrin. Imdacloprid belongs to insecticide class. It is most commonly used pesticide in Pakistan. That's why the residues of imidacloprid are most commonly present as compared to other pesticides. It was determined that leafy portion of vegetables contains most pesticides residues as compared to the pulpy portion of vegetable. If vegetables used after peeling then residues remain less in concentration Khan et al. (2011) [6]. Significant difference is present in concentration of outer portion and inner portion of vegetables. Bifenthrin is a pyrethroid pesticide. Bifenthrin residues were mostly determined in potato and spinach same as calculated by Asi et al. (2003) [2]. Carbofuran is a carbamate pesticide. When it spread in field then it becomes the part of vegetable and no pests can destroy the vegetables Fernandez et al. (2000) [3]. Chlorpyrifos belongs to organophosphate class of pesticides. From results it was concluded that chlorpyrifos calculated in high concentration in potato and radish samples. Significant difference is present in absorption of chlorpyrifos as compared to other pesticides; these results were also discussed by Asi et al. (2003) [2]. Quantitation of pesticides in this research project was done by measuring the diameter of standards spots along with different concentrations. It was studied that if concentration increases then diameter of spot can also increase and color brightness also increases. Then a standard curve was drawn between spot diameter versus concentration of pesticides. Samples diameter was compared with the standard diameter and concentration was calculated same as determined by the Asi et al. (2003) [2].

5.Conclusion

From this research study it was concluded that different pesticides possess different rate of adsorption in vegetables. No doubt, the pesticides degraded within days but their degraded elements remain in vegetables. Maximum residue limit is the lowest limit at which a pesticide can cause disease to human beings. Residue limit of pesticides should not exceed from maximum residue limit. Regular checking of vegetables collected from different markets should be done to analyze the concentration of different pesticides in vegetable. Different techniques can be used for detection and quantification of pesticides but high performance thin layer chromatography is a cheap method.

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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 and/or animal subjects (if exists) respect the specific regulations and standards.

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