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Evaluation of Multiple Pesticide Residues in Apple and Citrus Fruits, 1999–2001

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A balanced diet is essential for the maintenance of human health. Fresh fruits, their juices and other processed products are an important component of balanced diet, as they are rich sources of vitamins and minerals. Throughout the world fruit consumption is increasing day-by-day. Considering the demand of fruits in national and international markets, cultivation and production of fruit orchards in Pakistan has tremendously increased. Apple and citrus are the two most important fruits of Pakistan. These fruits are consumed in Pakistan and substantial quantity is exported as well. Fruit production is facing several problems, amongst them pest infection is an important part. Farmers follow modern production techniques and to ensure higher yields and returns from their orchards, they heavily spray pesticides, which leave behind residues on the produce. Awareness about pesticide residues and their implication in health disorders, teratogenicity and carcinogenicity of pesticides (Caffarelli et al. 1999) has got momentum throughout the globe.

Anastassiades et al. (1997, 1998 and 2000) analyzed multiple pesticide residues in citrus fruits from different countries and reported two methods for the determination of pre and post harvest pesticide residues in citrus fruits. Broglia (1999) determined pesticide residues in apple using GC, GC/MS and HPLC. Hirsotaka et al. (2001) reported multiple residue analysis of pesticides in vegetables and fruits using two-layered column with graphitized carbon and water absorbent polymer.

In continuation with our pesticides monitoring program for fruits and vegetables, present study was conducted to screen out pesticide residues used on apple and citrus fruits. Work was carried out in two phases (a) standardization of workable analytical methodology in apple and citrus fruits with known amounts of studied pesticides (b) monitoring of pesticide residues in different samples of apple and citrus fruits purchased from different selling points of Karachi, Pakistan.

MATERIALS AND METHODS

Ethyl acetate, n-hexane, toluene, sodium hydroxide (anhydrous), sodium sulphate (anhydrous) and activated graphitized charcoal were of analytical reagent grade

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and were purchased from Merck (Darmstadt, Germany). Florisil 60-100 US mesh was of analytical reagent grade purchased from BDH Laboratory Supplies (Poole, England). Methanol was of HPLC grade and was purchased from Merck (Darmstadt, Germany). Ultra pure water was prepared by passing the distilled water through Elgacan (High Wycombe Bucks England). Volac disposable pipettes were used as mini-columns.

A Shimadzu Model SPD-10A high performance liquid chromatograph (HPLC) was used. Light Source: Deuterium Lamp with changeable wavelength, Wavelength: 223 and 254 nm, Pressure: 2000 psi, Column: C-18 (ODS) – 15 cm x 6.0 mm i.d., stainless steel, Injection volume: 20 µl, Mobile phase: Methanol:Water (3:1) and Flow rate: 0.5 µL/min. were the operating conditions.

A Varian AG Model 3600 gas chromatograph (GC) equipped with Flame Ionization Detector (FID) data system Model DS-651, Thinkjet printer (Hewlet Packard) and Packed Glass column of 1.5% OV-17+1.95 OV-210 WHP (80-100 mesh)were used. Temperature of column oven: 230°C, Temperature of injector: 250°C, Temperature of detector: 300°C, Attenuation: 32, Range: 12, Gas flow rate of nitrogen: 13 ml/min, Gas flow rate of hydrogen: 4.5 ml/min and Gas Flow Rate of Air: 175 ml/min were the operating conditions.

Two hundred-seventy samples of apple and citrus fruits (Orange, Grapefruit, Lemon and Kino) were procured from different selling points of Karachi during 1999-2001. One kg of each was purchased in accordance with the standard procedure (FAO/WHO, 1982). Apple samples were sliced and homogenized while citrus fruits were peeled off and homogenized. Three sub-samples of 30 g were subjected to extraction, cleanup, identification and determination according to the procedure described below however extraction was carried out at the same day as the sampling was done.

Prior to monitoring studies, the efficiency of analytical methodology was evaluated in model experiments in which, homogenized samples of apple and citrus fruits were fortified with known quantities of each studied pesticide separately as well as in a mixture and were allowed to stand for three hours. These samples along with a control were then passed through the following procedure and finally were analyzed for percent recovery by HPLC and GC.

Primary evaluation of different solvents was made for extraction of multiple pesticides but a mixture of n-hexane:ethylacetate:toluene (1:1:3) was found to be the most suitable for extraction. 75 ml extracting mixture was added to each 30 g homogenized sub-sample, and the content was blended for 3 minutes. Sample was neutralized with sodium hydroxide solution, transferred to a stoppered conical flask, shaken mechanically on flask shaker for 3 hours and then was left in a deep freezer at -20 °C overnight. Next day the clear solution was decanted quickly and the extract was concentrated to 3 ml with a rotary vacuum evaporator at 60 °C. A mixture of activated charcoal and Florisil (1:10) was used for the cleanup step to

remove undesirable components from the sample extract. A Volac disposable glass Pasteur pipette was plugged with cotton, a small quantity of anhydrous sodium sulphate was poured into it and then 0.5 g mixture of activated charcoal and Florisil was added to it with continuous tapping. Little amount of anhydrous sodium sulphate was again added to the column so as to form a bed on the top of the column. The concentrated sample extract was poured into the column and the eluate was collected in a vial of 5 ml. Each eluate was evaporated to dryness at room temperature and then was added to it 2-5 ml methanol or 2 ml acetone for further analysis by HPLC or GC respectively.

Table 1. Percent recovery of studied pesticides in apple and citrus fruits.

S. No.	Pesticides Studied	Fortification Level (ppm)	Percent Recovery HPLC Mean*±SD	Percent Recovery GC(FID) Mean±SD
	Synthetic pyrethroids			
1	Bifenthrin	0.05	72.82±0.17	93.75±0.25
		0.10	87.32±0.05	98.32±0.51
2	Cypermethrin	0.05	70.98±0.10	97.85±0.09
		0.10	03.06±0.00	98.76±0.31
3	Deltamethrin	0.05	77.37±0.00	92.15±0.42
		0.10	90.92±0.21	96.74±0.13
4	Fenvalerate	0.05	71.38±0.34	98.81±0.28
		0.10	97.26±0.21	97.76±0.00
	Organophosphorous			
5	Ethion	0.01	99.03±0.08	---
		0.05	96.76±0.00	---
6	Methamidophos		62.02±0.12	88.92±0.00
		0.05	75.38±0.09	98.37±0.00
7	Monocrotophos	0.01	63.75±0.06	92.57±0.19
		0.05	80.56±0.00	97.96±0.23
8	Profenofos	0.01	76.98±0.27	96.02±0.36
		0.05	95.56±0.31	99.38±0.36
	Organochlorine			
9	Endosulfan	0.01	78.97±0.50	99.03±0.21
		0.05	97.32±0.07	99.81±0.43
	Carbamates			
10	Carbosulfan	0.01	92.89±0.31	---
		0.05	98.07±0.08	---
	Fungicides			
11	Benomyl	0.01	62.75±0.38	---
		0.05	93.68±0.38	---
12	Metalaxyl	0.01	91.73±0.15	---
		0.05	95.15±0.00	---
13	Thiabendazole	0.01	67.28±0.16	---
		0.05	96.78±0.84	---

Mean*: Mean of triplicate analysis of Apple / Citrus

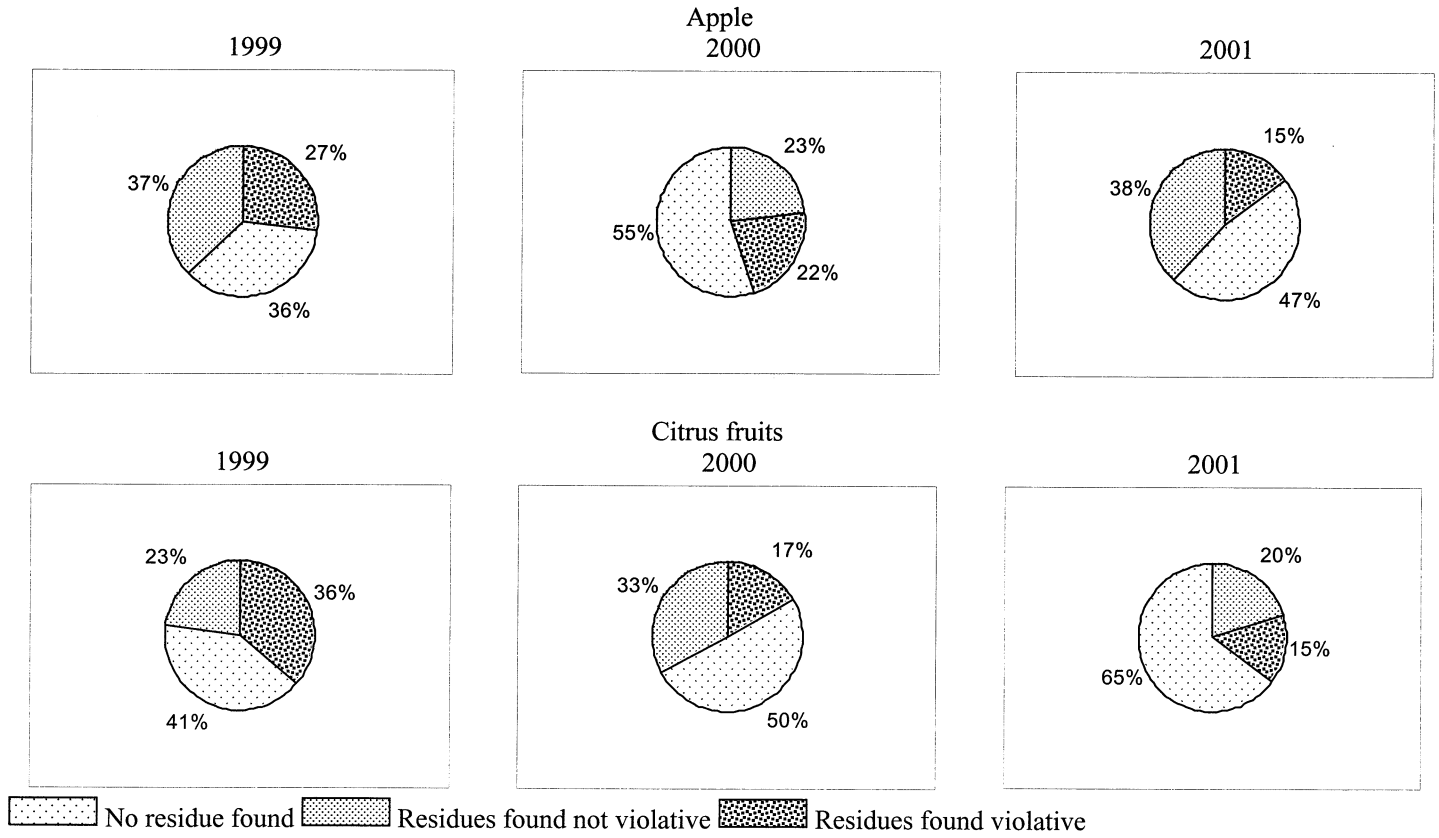

Figure 1. Distribution of pesticide residues in apple and citrus fruits.

Determination by HPLC was carried out with C-18 (ODS) column using a mixture of methanol and deionized water (3:1) as mobile phase at the rate of 0.5 ml/minute. Detection of fungicides and pyrethroids was carried out at the wavelength of 223 nm; while organophosphates were detected at the wavelength of 254 nm. Determination by GC was carried out with flame ionization detector. Detection of organophosphorus and pyrethroids was made in each cleaned up sample extract thrice together with relevant pesticide standard in acetone using 0.1-0.5 µg injection. Control sample processed in a similar manner did not show any interfering peak that might be attributed to studied pesticides. Percent recovery of the studied pesticides is presented in Table 1. Results of 270 samples

Table 2. Evaluation of multiple pesticide residues in apple and citrus fruits by frequency of occurrence in 270 samples 1999-2001.

S. No.	Analyte found	No. of samples contam-inated		Qty[1]. found (ppm)	No. of samples exceeded MRLs		MRLs of pesticides	
		A[2]	C[3]		A	C	A	C
	Synthetic pyrethroids							
1	Bifenthrin	15	08	0.01-7.45	07	03	0.5	1.0
2	Cyfluthrin	06	02	0.49-3.25	03	02	1.0	2.0
3	Cyhalothrin	05	01	0.05-2.07	01	01	0.4	10.0
4	Cypermethrin	11	01	Traces-5.56	01	04	20.0	2.0
5	Fenpropathrin	03	04	0.01-10.05	-	-	-	-
6	Fenvalerate	18	05	Traces-5.68	11	01	2.0	2.0
	Organophosphorous							
7	Chlorpyrifos	10	11	0.02-13.22	03	04	1.0	0.3
8	Methamidophos	01	01	4.63-0.32	-	-	-	-
9	Methylparathion	02	01	0.24-0.64	02	01	0.2	0.2
10	Monocrotophos	04	02	1.44-7.19	-	03	-	0.2
11	Profenofos	11	09	0.13-15.62	-	05	-	1.0
12	Quinalphos	01	-	0.32	01	-	0.02	0.8
	Organochlorine							
13	Endosulfan	11	16	0.05-13.07	-	-	-	-
14	Dicofol	07	16	0.05-13.97	01	03	3.0	5.0
15	Carbamates							
16	Carbofuron	09	05	1.66-5.21	-	-	-	-
17	Carbosulfan	02	13	-	-		-	-
	Miscellaneous group							
17	Etion	-	42	Traces-8.86	-	05	-	5.0
18	Propargite	12	-	Traces-0.45	-	80	40.0	-
19	Fungicides							
20	Benomyl	13	23	Traces-20.15	04	04	5.0	10.0
21	Metalaxyl	07	-	Traces-5.75	04	01	0.05	5.0
22	Thiabendazole	16	17	Traces12.52	02	01	10.0	10.0

[1]Quantity, [2]Apple and [3]Citrus

are presented in Table 2.

RESULTS AND DISSCUSSION

A comparative picture of analytical data for three years apple and citrus fruit samples (Table 2) shows that synthetic pyrethroids and organophosphate were the main contaminants in apple and citrus fruits. Apple samples were contaminated with more than one of the enlisted pesticides bifenthrin, cyfluthrin, cypermethrin, fenvalerate, chlorpyrifos, monocrotophos, profenofos, endosulfan, carbosulfan, ethion, propargite, benomyl, thiabendazole and metalaxyl. In case of citrus fruits bifenthrin, cypermethrin, fenpropathrin, fenvalerate, chlorpyrifos, profenofos, endosulfan, dicofol, carbosulfan, carbofuran, benomyl and thiabendazole were frequently found.

Year-wise distribution of pesticides contamination in citrus and apple from 1999-2001 (Figure 1), clearly shows that there is marked reduction in number of samples contaminated with pesticide residues as well as the number of samples exceeding MRLs as given by WHO (FAO/WHO, 2000). This trend might be explained in terms of grower's awareness about judicious use of pesticides.

REFERENCES

Anastassiades M, Scherboum E (1997) Multiresidue method for determination of pesticide residues in citrus fruits by GC-MSD Part-2. Analysis of citrus fruits of different origion. Dtsch Lebensm-Rundsch 93: 393-396.

Anastassiades M, Scherboum E (1998) Multiresidue method for the determination of pesticide residues in citrus fruits by GC-MSD Part-3. Determination of 2, 4-D after alkalie treatment." Dtsch Lebensm-Rundsch 94: 45-49

Anastassiades M, Scherbaum E, Schewakck W (2000) Multiresidue method for the determination of pre- and post-harvest pesticides in citrus fruits part-4: determination of organotin pesticides. Dtsch Lebensm-Rundsch 96:466-477

Broglia L, Fonte A, Gavio, Maria T, Ghioni V (1999) Post-harvest treatments; determination of diphenyl-amine, ethoxyquine, thiabendazole and carbendazim residues in apples. CA Selects Insecticides, Issue 22: 15

Caffarelli V, Rapagrani MR, Letardi A, Lancia B, Santaroni P (1999) Pesticide residues in fruit and vegetable products and the carcinogenic risk for consumers. Riv Sci Aliment 28: 11-24

FAO/WHO (1982) Food Standards Programme Codex Alinorm 79/24 Appendix Annex Technical Monograph

FAO/WHO Codex Alimentarius Commission Pesticide Residues in Food, Vol. 2B-2000, Second Edition Revised

Hirotaka O, Kazuhio A, Masahiro O, Shinjiro H (2001) Multiresidue analysis of pesticides in vegetables and fruits using two-layered column with graphitized carbon and water absorbent polymer. Analyst 126: 1529-1534

Yamazaki Y, Ninomiya T (1999) Determination of benomyl, diphenyl, o-phenyl-phenol, thiabendazole, chlorpyrifos, methidathion and methylparathion in

oranges by solid-phase extraction, liquid chromatography and gas chromatography. JAOAC Int 82: 1474-1478

Zahida P, Masud S Z (2002) Fungicide residues in apple and citrus fruits after post-harvest treatment. Pakistan J Sci Ind Res 45: 246-249