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DETERMINATION OF PESTICIDE RESIDUES IN FRUITS OF NAWABSHAH DISTRICT, SINDH*, PAKISTAN

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Abstract

Eight fruit samples of apple, guava, orange, grapes, pear, persimmon, banana and pear purchased from the local markets of Nawabshah district, Sindh and residues of pesticide of organophosphate (OP), pyrethroid and organochlorine (OC) (i.e., dichlorvos, fenvalerate, dimethoate, methyl parathion, fenitrothion, cypermethrin, endosulfan, deltamethrin, mevinphos, chlorpyriphos, profenofos and dicofol) were monitored in fruit samples by Gas Chromatography (GC). All the fruit samples were found contaminated except banana and among these only apple samples were found exceeding the maximum residue limits (MRL) of Codex Alimentarius Commission.

Introduction

Apple, guava, grapes, orange, pear, persimmon banana and peach form the main fruit component of the diet of an average citizen of Pakistan. Moreover, the important fruits are grown in abundance (4.7 million tons) in Pakistan and also exported to other countries of the world. The share of food is about 13.2% in total exports including fruits (Anon., 2008). Pesticide residues in food commodities are a fast growing global problem with serious repercussions on human health (Asmatullah, 1996). Furthermore, irrational use of pesticides has created new pests that have never been a problem before (Huque, 1990). The resurgence of pests, development of resistance (Zahoor, 1999), contamination of soil, water and air, destruction of predators, parasites and other non-target organisms including wildlife, brought about the realization that these chemicals are not exclusively doing the job they were intended to do but also have adverse effects on ecological systems with which human welfare is inseparably bound (Hussain, 1999). The dependency on pesticides is evident from the increasing trend in its consumption from 665 mt in 1980 to 45,680 mt in 1999 (Anon., 2000) and recently reduced to 25000 mt in 2006 (Anon., 2007), contrary to the experience in industrialized countries like USA, where herbicides make up 85% of total pesticides (Anon., 1987). At present more than108 types of insecticides, 30 types of fungicides, 39 types of weedicides, 5 types of acaricides and 6 different types of rodenticides are being used in Pakistan (Anon., 2002) and the import of pesticides has decreased from 41,406 tons in 2003-04 to 20394 tons in 2006-07. The misuse of pesticides has led to tremendous economic losses and hazards to human health. Human exposure to pesticides is usually estimated by measuring the levels in the environment i.e., soil, water and food (Tahir *et al*., 2001, Ahmad 2004, Anwar *et al.,* 2004, 2005, Ahmad *et al*., 2007). About 60-70% of pesticide poisoning cases were reported due to occupational exposure and female cotton pickers appeared to be at high risk of hazards (Ahmad, 1998; Tahir, 2000) and recently Tariq *et al*., (2007) reviewed the pesticides poisoning cases in Pakistan. The present work was undertaken, to provide base-line data on pesticide residues level in fruit samples for determination of toxicity level of pesticides in fruits for the development of national codex standards.

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Materials and Methods

All the fruits samples were collected randomly from the selected districts of Nawabshah, Sindh. One kilogram each of fruits (i.e., apple, guava, oranges, grapes, pear, persimmon, banana and peach) collected from the local markets was transported in ice box to the laboratory. These samples were chopped, sub-sampled and preserved in a freezer till further processing. The method of Tahir *et al*., (2001) was followed for extraction and cleanup of samples.

One kg of the sample was chopped and mixed thoroughly. A subsample of 25 gm was taken out and blended with 50 ml of acetone, 50 gm of anhydrous Sodium sulphate and 50 ml of a mixture of Cyclohexane and ethylacetate (1:1). The mixture was allowed to stand for some time until a clear supernatant was formed and 30 ml supernatant was taken into a round bottom flask. A few drops of 10% propandiol in ethylacetate and about 4-6 glass beads were added. The solvent was evaporated at 40°C under vacuum in rotary evaporator. The contents were reconstituted in 6 ml of cyclohexane and ethylacetate (1:1) and then passed through high-flow super cells. Two ml of this sample was applied on GPC column for further cleanup. After passing through GPC column, the samples were dried under vacuum and reconstituted in 1ml ethylacetate for analysis on Gas Chromatograph (GC). The retention time was within ± 2% of that of the standard. The method validation consisted of three sample sets. Each set included three levels of fortification (0.01, 0.05 and 1.0 ppb) and a method blank. All spikes and method blank samples were processed through the entire analytical method. Quantification was based on external standard calculation using the peak area.

Gas Chromatograph, Perkin-Elmer, Autosystem, Microprocessor was fitted with Electron Capture Detector (ECD-Ni^{63}) and Nitrogen Phosphorous Detector (NPD). Nitrogen and Air Generator Peak Scientific. Hydrogen Generator, Peak Scientific, Gel Permeation Chromatograph (GPC), Mikrolab Arhus A/S, USA. Rotary Evaporator, made Buchi R-114/A, Switzerland. Food Blender, Germany. FlASK Shaker SF1, Sartatorius single pan analytical balance and Refrigerator/Freezer.

The analytical grade standards of insecticides (dichlorvos, fenvalerate, dimethoate, methyl parathion, fenitrothion, cypermethrin, endosulfan, deltamethrin, mevinphos, dicofol, chlorpyriphos and profenofos were purchased form Riedel-d Haen AG seelze, Germany or obtained from other institutes of Pakistan. Stock solutions and required working dilutions were prepared in ethylacetate. All other solvents and reagents were of extra pure GC/HPLC grade. Acetone (Merk) Ethylacetate (Merk), cyclohexane (BDH) and n-Hexane (BDH). Anhydrous sodium sulphate (Merk), dichloromethane (Lock-light Ltd.), propane 1, 2-diol (Pharmacos Ltd., UK), high flow super cells (BDH) bio-beads, SX3 200-400 (Reidel-de Haën), sodium hydroxide (Merck), potassium dichromate (Merck), sodium chloride (Merck) and Millipore distilled water.

Results and Discussion

Pesticide residues were determined in eight fruit samples of apple, guava, grapes, orange, pear, persimmon, banana and peach procured from the local markets of Nawabshah, district, Sindh by capillary gas chromatograph equipped with ECD (Table 1). Endosulfan was determined in the quantity of 0.774 mg Kg^{-1} and 0.004 mg Kg^{-1} in apple and peach. Dimethoate was recorded in the quantity of 0.042 mg Kg^{-1}, 0.090 mg Kg^{-1}, 0.139, mg Kg^{-1} 0.003 mg Kg^{-1}, 0.042 mg Kg^{-1} in apple, guava, orange, pear and persimmon

respectively. Chlorpyrifos was determined in the quantity of 0.038 mg Kg^{-1}, 0.020 mg Kg^{-1} and 0.055 mg Kg^{-1} respectively in apple, guava, pear and persimmon respectively. Methyl parathion was found only in the quantity of 0.0442 mg Kg^{-1} in guava. Fenitrothion was recorded in the quantity of 0.024 mg Kg^{-1}, 0.003 mg Kg^{-1}, 0.003 mg Kg^{-1} and 0.030 mg Kg^{-1} in apple, guava, persimmon and peach respectively. Cypermethrin was found in the quantity of 0.940 mg Kg^{-1}, 0.030 mg $Kg^{-1}$and 0.031, mg Kg^{-1} in apple, persimmon and peach respectively. Fenvalerate was found in the quantity of 0.055 mg Kg^{-1}, 0.002 mg Kg^{-1}, 0.021, mg Kg^{-1} 0.021 mg Kg^{-1} and 0.455 mg Kg^{-1} in guava, orange, persimmon and peach respectively. Deltamethrin was recorded in the quantity of 0.039 mg Kg^{-1}, 0.020 mg Kg^{-1} and 0.005 mg Kg^{-1} in apple, guava and pear respectively.

A total of seven fruit samples were found contaminated with pesticides and among those only one out of eight fruit samples was found above the MRL. Multiple pesticide residues were found in the samples. Four fruits analyzed were found to be contaminated with 1-2 residues of the component pesticides tested while five samples were found contaminated with 5-7 pesticide residues. Most of the fruits (5) were found contaminated with dimethoate residues with a mean value of 0.04 mg Kg^{-1}. The fenitrothion and fenvalerate were detected in four samples followed by cypermethrin and deltamethrin in three samples, endosulfan in two and methyl parathion in one sample while dicofol was not detected in any sample. Only apple sample (0.94 mg Kg^{-1}) was found containing cypermethrin residues above the MRL (0.1 mg Kg^{-1}). The total residue of pesticides was also found highest in apple $\sum$0.824 mg Kg^{-1} followed by guava $\sum$0.25 mg Kg^{-1}, persimmon $\sum$0.148 mg Kg^{-1} and orange $\sum$0.141 mg Kg^{-1} while other fruits were found to contain $\sum$residues in the range of 0.005-0.086 mg Kg^{-1} exceeding the MRL set by the FAO only for individual pesticides in a sample and not for the cumulative residues calculated in the study.

Pesticides use has no doubt increased the agricultural production in general but persistent residues of these chemicals have tremendous harmful impact on the environment and also on human health. A considerable attention has been focused on the threat to human life coming from the dietary food, drinking water, and the residential risk caused by the presence of pesticide residues. Pesticides and their contamination of food products are regulated through some concept like Maximum Residue Limits (MRLs), Average daily intake (ADIs) and Good Agricultural Practices (GAPs). Data from nutritional surveys, reflects details of the regional diet patterns and quantum of a need when estimating the daily intake of any given pesticide through food. These parameters, ADI, terminal residues and diet patterns are critical inputs needed to derive the MRLs of pesticides in food commodities.

The objective of risk assessment from the point of view of food safety is to ensure that the sum total of pesticide residues in the total diet dose not exceed ADI, even after taking into account the possible exposure through other sources. The Codex Alimentations Commission of the Food and Agriculture Organization (FAO) of the United Nation and WHO (Anon., 2004) have recommended respect of MRL in fruits and vegetables. Monitoring of pesticide residues is a key tool for ensuring conformity with regulation and providing a check on compliance with Good Agricultural Practice. The consideration of possible exposure to pesticide residues is an integral part of the risk assessment process to ensure that the ADI of the pesticides are not exceeded. As long as the residue of the pesticides ingested by consumers does not exceed the corresponding ADI consumers are considered to be adequately protected. This is useful for assessing human exposure to pesticides through the food supply and for understanding the magnitude of health risk.

Table 1 Pesticide residues (mg kg^{-1}) in fruit samples collected from the markets of Nawabshah district, Sindh, Pakistan.

S. No.	Fruits	Pesticides									
		Endosulfan	Dimethoate	Chlorpyrifos	Methyl parathion	Fenitrothion	Cypermethrin	Fenvalerate	Deltamethrin	Dicofol	Total
1.	*Apple*	0.774	0.047	0	0	0.024	0.94	0	0.039	0	1.824
2.	*Guava*	0	0.09	0.038	0.0442	0.003	0	0.055	0.02	0	0.2502
3.	*Orange*	0	0.139	0	0	0	0	0.002	0	0	0.141
4.	*Grapes*	0	0	0	0	0	0	0	0.005	0	0.005
5.	*Pear*	0	0.003	0.02	0	0.003	0	0	0	0	0.026
6.	*Persimmon*	0	0.042	0.055	0	0	0.03	0.021	0	0	0.148
7.	*Banana*	0	0	0	0	0	0	0	0	0	0
8.	*Peach*	0.004	0	0	0	0.03	0.031	0.021	0	0	0.086
	Mean	0.097	0.040	0.014	0.006	0.008	0.125	0.012	0.008	0.000	0.310
	STD	0.273	0.051	0.022	0.016	0.012	0.330	0.020	0.014	0.000	0.618
	95%C	0.006	0.001	0.0005	0.0004	0.0003	0.007	0.0004	0.0003	0	0.014

Note: Values are the mean of three samples analyzed in duplicate collected form the markets of three locations (1x2x3) i.e., Nawabshah, Sakrund and Daulatpur. Whereas, 0 stands for "pesticide not detected" and it is included in calculating the mean.

In the present study eight kinds of fruit samples (apple, guava, orange, grapes, pear, persimmon, banana and peach) colleted from Nawabshah, Bahawalpur and Lodhran were analyzed for endosulfan, dimethoate, chlorpyrifos, methyl parathion, fenitrothion, cypermethrin, fenvalerate, deltamethrin and dicofol. Only one i.e., apple sample, out of eight fruits was found containing cypermethrin above MRL collected from Nawabshah.

In the developed countries many reports are available on the monitoring of pesticide residues in fruits and vegetables detected above MRLs (Atuma, 1985; Kawamura *et al*., 1986; Dogheim *et al*., 1999; Reddy *et al*., 1918; Blasco, *et al*., 2005, Cesnik *et al*., 2006 and Zawiyah *et al*., 2007). The findings of the present study verify the presence of pesticides (cypermethrin, dimethoate, chlorpyriphos and fenitrothion) in fruit samples which are used in pre- harvest treatment with different applications that cover a wide range of pests and diseases of fruit. The results of the present study are consistent with the observations previously reported for pesticide residues in fruits of Canada (Frank *et al*., 1987), India (Kawamura, *et al*., 1986), Egypt (Dogheim *et al*., 1996 &1999) and Spain (Blasco *et al*., 2005 &2006).

Perusal of the contaminated data on pesticides in samples of fruit in Pakistan indicates their presence an alarming situation. The pesticide residues have been reported in different fruits at different intervals throughout the country (Tahir *et al.*, 2001; Parveen *et al*., 2004 & 2005; Ahmad, 2004; Anwar, *et al*., 2004; Hussain *et al*., 2004 and Hassan, *et al*., 2007). The samples analyzed were mostly found contaminated with pesticides which are in full support of the present results. The overall pesticide consumption in the country has decreased from 41406 tons in 2003-04 to 20394 tons in 2006-2007 (Anon., 2007). Decrease in number of samples not exceeding MRLs can be linked with decrease in quantity of pesticide consumption. To prevent adverse effects on public health it is a must to establish control measures in order to ensure MRLs to be respected (Ahmad, 2004). Recently pesticide residues in fruit samples collected from farmer's field under supervised trials were analyzed (Khan, 2005). In Pakistan no attempts has been made to calculate the residues on agricultural commodities under Good Agricultural Practice. Monitoring of pesticide residues should be legalized in developing countries like Pakistan to face the implication of WTO (Anwar, 2006) and to control the indiscriminate use of the pesticides in the country and to ensure that the consumers are not exposed to any risk by eating food containing pesticide residues.

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