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Organophosphate Pesticide Residues in Okra and Brinjal Grown in Peri-Urban Environment of Big Cities of Punjab

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Summary: Peri urban farming system is main supplier of vegetables to urban population and organophosphate pesticides exposure due to consumption of okra and brinjal to urban population has been estimated in this study. Okra and brinjal samples (90 samples for each vegetable) were collected from peri-urban farming area of Faisalabad, Multan and Gujranwala and were analysed by GC-ECD for quantification of chlorpyrifos, profenophos and triazophos residues. Data were statistically analyzed to calculate, mean and standard deviation. Quantification of pesticides residues revealed that 85 (47.22%) samples were contaminated and 15 (8.33 % of total samples and 17.64% of contaminated) samples showed residues value higher than MRL'S. Out of 85 contaminated samples 27 (31.76%) samples were contaminated with chlorpyrifos, 30 (35.29%) samples with profenofos and 29 (34.11) were contaminated with triazophos. The mean concentration of chlorpyrifos in okra was 0.192 mg Kg^{-1} and brinjal 0.197 mg Kg^{-1}. The mean concentration for profenofos in okra was 0.02 mg Kg^{-1} and brinjal 0.035 mg Kg^{-1}. Similarly mean concentration for triazophos in okra was 0.009 mg Kg^{-1} and brinjal 0.01 mg Kg^{-1}. Overall, 6.67% samples of okra and 6.66% samples of brinjal contaminated with chlorpyrifos exceeded EU MRL of 0.05 mg kg^{-1}. Okra samples were found contaminated at frequency of 4.44% and brinjal samples at frequency of 5.56% with higher profenofos residues level than MRL of 0.05 mg kg^{-1}. Approximately, 6.67% samples of okra and 5.6% samples of brinjal had profenofos residue level above MRL of 0.01 mg kg^{-1}. This study may be helpful for building database regarding regional exposure of urban population and facilitate in estimation of possible risk to their health in our daily life.

Keywords: Pesticides, Organophosphate, Okra, Brinjal

Introduction

Vegetables enclose priceless nutritional characteristics, which are supportive in repairing and appropriate growth of human body. Conversely, they can also be a source of toxic contaminants that are formulated to control pests in crops known as pesticides [1]. Contamination of crops and environment is directly associated to agro-chemical applications, industrial and domestic discharges [2]. Pesticides can be herbicides, insecticides and fungicides depending on the target pests. Based on the composition and mode of action, insecticides are further classified into organophosphate, organochlorine, carbamates, pyrethroids, and neonicotinoids. Among various pesticide classes, organophosphorus pesticide (OPPs) group is the most widely used class of agricultural pesticides to increase world food production [3,4].

Chlorpyrifos is used all over the world on wide range of crops and vegetables especially okra and brinjal. It is one of the most stable and persistent member of OPPs in comparison with others [5]. Due to different metabolism of propylthiol group in mammals, Profenofos is more lethal to insects as compared to mammals. Whereas Triazophos is a systemic and broad-spectrum organophosphate pesticide sprayed now a days, it is considered to be reasonably toxic in nature and is most effective against the attack of nematodes on vegetables [6]. Besides, OPPs chemical residues of some pyrethroid in brinjal and okra fruits have also been reported by Zafar *et al.,* [7].

During the entire growth period of vegetables and crops especially okra and brinjal, farmers apply a large amount of organophosphate pesticides to get higher yields. This is believed to be one of the major reasons of higher values of pesticides residues in okra and brinjal [8]. Besides their effective control of insect pest attack at the same time they are dangerous for human health and surroundings [9]. Ingestion of such highly contaminated vegetables either in raw or processed form by consumer may result in some serious health hazards [10]. Regarding human health such large quantity of sprays per crop indicates an alarming condition [11]. OPPs vapors are capable of penetrating the respiratory epithelium, cause cornea and skin complications [12]. OPPs are considered to be carcinogenic, mutagenic, genotoxic, cytotoxic, immunotoxic and teratogenic) [13].

Vegetables supplied to cities markets are taken from peri urban farming system as their growers are at shorter distance. Farmers use pesticides on vegetables and market them without giving stay time. This practice may increase chances of higher pesticides residues exposure to end consumer. This study was designed to estimate possible risk of OPPs residues exposure to urban population consuming okra and brinjal. Samples were collected from peri-urban farming system of big cities of Pakistan including Faisalabad, Gujranwala and Multan. The residual level of OPPs residues (chlorpyrifos, profenofos and triazophos) in brinjal and okra samples was quantified and compared the levels of OPPs residues with their maximum residual limits (MRLs).

Results and Discussion

In this study, 3 pesticides profenofos, chlorpyrifos and triazophos in okra and brinjal were analyzed. Samples were taken from 3 highly populated cities of the Punjab province in Pakistan viz: Faisalabad, Multan and Gujranwala. Total 180 samples were taken with frequency of 30 samples of each vegetable from each city for possible presence of each pesticide (Table-2).

Okra and brinjal (30 samples for each vegetable) were analyzed for each pesticide residue i.e. chlorpyrifos, profenofos and triazophos.

Occurrence of Chlorpyrifos

Mean residue levels for detectable chlorpyrifos in okra and brinjal were as follow: Faisalabad, 0.2878 mg kg^{-1} and 0.1713 mg kg^{-1}; Gujranwala, 0.1220 mg kg^{-1} and 0.1306 mg kg^{-1}; Multan, 0.1682 mg kg^{-1} and 0.2886 mg kg^{-1}. Overall 30%, 23.33%, 33.33% samples of okra and 26.67%, 33.33%, 43.33% samples of brinjal were found contaminated with chlorpyrifos (Table-1). It was calculated that 9, 7 and 11 samples of okra; 8, 10 and 13 samples of brinjal had residues of chlorpyrifos procured from peri-urban areas of Faisalabad, Gujranwala and Multan, respectively. Chlorpyrifos residues analysis revealed that 3, 1 and 2 samples of okra violated the recommended MRL (0.5 mg kg^{-1}). Similarly 1, 1, and 4 samples of brinjal exceeded the MRL 0.5 mg kg^{-1} (Table-2). The data showed only few samples out of contaminated were above the MRL established by the EU, however their presence even in a few samples claims significantly alarming situation for end consumer, with the reason of their high toxic effect on human health. The mean concentration of chlorpyrifos in okra was 0.192 mg Kg^{-1} and in brinjal it was 0.197 mg Kg^{-1} (Table-1). The literature showed the mean concentration of chlorpyrifos in brinjal 0.024 mg kg^{-1} and okra 0.0024 mg kg^{-1} [14]. Randhawa *et al.,* [15] studied that chlorpyrifos residues were found in okra (0.021–1.930 mg kg^{-1}) and okra (0.040–0.850 mg kg^{-1}).

Table 1: Organophosphate residues in okra and brinjal in different cities of Punjab

Pesticides	Area	Vegetable						MRLs (mg Kg-1)
		Okra			Brinjal			
		Range (mg Kg-1)	Mean±SD (mg Kg^{-1})	C.R (%)	Range	Mean+SD (mg Kg-1)	C.R (%)	
Chlorpyrifos	Faisalabad	0.05-0.7	0.2878±0.034	30	0.01-0.6	0.1713±0.024	26.67	0.5
	Gujranwala	0.003-0.7	0.1220±0.025	23.33	0.01-0.8	0.1306±0.023	33.33	
	Multan	0.01-0.9	0.1682±0.029	36.67	0.001-1.1	0.2886±0.040	43.33	
Profenofos	Faisalabad	0.001-0.06	0.0170±0.001	33.33	0.01-0.07	0.0384±0.002	23.33	0.05
	Gujranwala	0.01-0.07	0.0277±0.002	23.33	0.01-0.06	0.0345±0.002	13.33	
	Multan	0.001-0.08	0.0161±0.002	43.33	0.01-0.1	0.0314±0.002	36.67	
Triazophos	Faisalabad	0.001-0.017	0.0069±0.0005	30	0.003-0.017	0.0066±0.00539	20	0.01
	Gujranwala	0.001-0.014	0.0082±0.0006	26.67	0.001-.018	0.0074±0.0006	30	
	Multan	0.001-0.008	0.0147±0.002	40	0.0003-0.021	0.0168±0.003	36.67	

C.R = Contamination Rate MRLs = Maximum Residual Limits

Table-2: Organophosphate residues in okra and brinjal with %MRL.

Pesticides	Area	Vegetable									
		Okra					Brinjal				
		n	Cont.	N.D	>MRL	>MRL (%)	n	Cont.	N.D	>MRL	>MRL (%)
Chlorpyrifos	Faisalabad	30	9	21	3	10	30	8	22	1	3.33
	Gujranwala	30	7	23	1	3.33	30	10	20	1	3.33
	Multan	30	11	19	2	6.67	30	13	17	4	13.33
Profenofos	Faisalabad	30	10	20	1	3.33	30	7	23	2	6.67
	Gujranwala	30	7	23	1	3.33	30	4	26	1	3.33
	Multan	30	13	17	2	6.67	30	11	19	2	6.67
Triazophos	Faisalabad	30	9	21	2	6.67	30	6	24	1	3.33
	Gujranwala	30	8	22	1	3.33	30	9	21	1	3.33
	Multan	30	12	18	3	10	30	11	19	3	10

n= Total Sample; N.D= Not Detected; Cont.= Contaminated; MRL= Maximum Residual Limit

Occurrence of Profenofos

Analysis of okra and brinjal samples was carried out in order to investigate residues of profenofos procured from peri-urban surroundings. Mean residue levels for detectable profenofos in okra and brinjal were as follow: Faisalabad, 0.01700 mg kg^{-1} and 0.03843 mg kg^{-1}; Gujranwala, 0.02771 mg kg^{-1} and 0.03450 mg kg^{-1}; Multan, 0.01611 mg kg^{-1} and 0.03145 mg $kg^{-1,}$ respectively.

Overall 33.33%, 23.33%, 43.33% samples of okra and 23.33%, 13.33%, 36.67% samples of brinjal showed the presence of profenofos (Table-1). Profenofos was detected for 10, 7 and 13 samples of okra; 7, 4 and 11 samples of brinjal had positive detection for profenofos obtained from peri-urban areas of Faisalabad, Gujranwala and Multan, respectively. For profenofos 1, 1 and 2 samples of okra exceeded the recommended MRL (0.05 mg kg^{-1}) likewise 2, 1 and 2 samples of brinjal violated the MRL 0.5 mg kg^{-1} (Table-2). The mean concentration for profenofos was in okra 0.02 mg Kg^{-1} and brinjal 0.035 mg Kg^{-1} (Table-1). In the previous study, Ramadan [16] reported that the mean concentration of profenofos in/on okra in supervised field was 4.50 mg Kg^{-1}, harvested same day of profenofos (700 ml/acre) spray. In a field study profenofos residues 3.21 mg Kg^{-1} was detected in okra harvested very next day of spray with profenofos as active ingredient at the rate 400 ml/ acre [17].

Occurrence of Triazophos

Samples analyzed for triazophos residues showed the scenario of detectable residues as: mean residue levels for detectable triazophos in okra and brinjal in the samples from Faisalabad, 0.00691 mg kg^{-1} and 0.00667 mg kg^{-1}; Gujranwala, 0.00819 mg kg^{-1} and 0.00742 mg kg^{-1}; Multan, 0.01478 mg kg^{-1} and 0.01686 mg kg^{-1} respectively. On an average 30%, 26.67%, 40% samples of okra and 20%, 30%, 36.67% samples of brinjal had the residues of triazophos (Table-1). Triazophos was detected in 9, 8 and 12 samples of okra; 6, 9 and 11 samples of brinjal were contaminated with triazophos collected from peri-urban areas of Faisalabad, Gujranwala and Multan, respectively. For triazophos 2, 1 and 3 samples of okra violated the recommended MRL (0.01 mg kg^{-1}). Similarly 1, 1, and 3 samples of brinjal exceeded the MRL 0.01 mg kg^{-1} (Table-2). Similarly, mean concentration for triazophos in okra was 0.009 mg Kg^{-1} and brinjal 0.01 mg Kg^{-1} (Table-1). In past studies the mean concentration of triazophos in brinjal was 0.0008 mg Kg^{-1} and in okra it was 0.0024 mg Kg^{-1} [14].

Vegetables are crops of short duration. It is necessary to spray a pesticide on crop plants repeatedly during the entire period of growth and sometimes even at the harvesting stage in subtropical climate prevailing during growth and maturity of vegetables [10]. This contamination of pesticides causes toxicity to health and environment, due to which progressed countries have banned use of these chemicals but they are still in use in the developing countries. In Pakistan large number of pesticides is used to control pests which resulted in contamination of soil and ground water [18]. As a result, vegetables grown in/on contaminated soils can have pesticides residues from soil and ground water. In Pakistan, farmers use pesticides in order to increase productivity without taking care of their toxic effect on human health and environment. Indiscriminate use of pesticides on vegetables and are transfer to market very next day of spray; are reasons for high pesticides residues. This risk of pesticide exposure can be minimized by integrated pest management, pest scouting and stay time should be given after spray before marketing. This study provides pesticides residues status in vegetables in major cities of Punjab Pakistan, which may be helpful for Government in conducting screening survey of vegetables in major cities of Pakistan to establish a defined law for pesticides use on crops to minimize health risk to human life.

Experimental

Selection of Field

Thirty farmers, using pesticides as per their normal schedule, growing vegetables (brinjal and okra) in the peri-urban surroundings of districts Faisalabad, Gujranwala and Multan were randomly selected. Farmers were selected on the base of their distance from the city markets and their produce destination. Those farmers were selected which had their farming system within radius of 10 Km from the main vegetable market of respective city. Preference was given to those farmers who were closer to city with the reason to assure that their produce are marketed to the nearest respective market.

Collection of Samples

Samples were taken from 5 different places of selected farm 3 from each side and 3 from center with maximum care to reduce error and have representative sample. Thirty samples of each vegetable, at optimum maturity stage, were procured. They were kept in polyethylene air tight bags and transported to laboratory for immediate analysis followed by sample preparation, extraction etc. Those samples, which were not immediately analyzed due to any reason, were stored at -40°C after extraction process until their analysis to avoid any degradation of pesticide residues during ordinary storage.

Extraction of Residues

Residues of OPPs were extracted from the homogenized sample using the method of Kadenezki *et al.,* [19] with some modifications illustrated by Khan *et al.,* [20]. Ethyl acetate, an extracting solvent, was added to blended 1 kg of chopped sample. According to this method 50 gm of homogenized sample was taken in 250 mL Erlenmeyer flask. 20 gm anhydrous sodium sulphate (Analytical grade) was added and mixed in homogenized vegetable sample in flask to prevent the clot formation. 10 mL saturated sodium chloride was added in the mixture. 70 mL ethyl acetate (HPLC grade) was added in the sample. Glass beads were added in mixture to facilitate the extraction process. Mixture in flask was shaken at speed of 240-250 rpm on a horizontal mechanical shaker for time period of 1 hour. The extract was filtered using Whatman (No. 4) filter paper, collected in inert plastic bottle and stored at -40°C prior to further analysis.

Clean-up of Filtered Extract

Pesticides residues analysis requires high sensitivity as these are present in traces. Therefore, to get high sensitivity, cleanup operation was carried out so that interfering substances in the extract could be removed and precise measurement of residues could be done. For this reason, residues of pesticides were cleaned up using column chromatographic technique as reported by Kumari *et al.* [21].

Column was prepared by using glass wool, anhydrous sodium sulphate, activated silica gel and charcoal. After washing of the prepared column with acetone (HPLC grade) loading of sample was done and the extract was eluted using 50 mL of acetone and hexane mixture (3:7 V/V) at the rate of 1 mL min^{-1}. The cleaned up elute was received in round bottom flask and concentrated in rotary evaporator at 40°C to final volume of 1-1.5 mL. The concentrated elute was transferred to small vials of volume 1.5 mL and then completely dried by gentle nitrogen stream.

GC-ECD Analysis

The cleaned extracts were analyzed on Agilent GC model 6890 equipped with capillary column using electron capture detector (ECD) as reported by Chandra *et al.,* [22]. The separation of pesticides was done HP-5 MS column. Nitrogen was used as the carrier gas at 9.6 psi pressure and total flow of gas was maintained at 60 mL min^{-1}. Gas flow in the column was maintained at the rate of 2 mL min^{-1}. The injector and detector temperatures were set at 270°C and 300°C, respectively. The oven conditions were programmed as follow: initial temperature 100°C for 2 min; increase up to 220°C at a rate of 6°C/min held for 2 min, increase temperature up to 260°C at a rate of 8°C/min held for 2 min and final increase of temperature up to 300°C at a rate of 10°C/min held for 10 min. The injection volume of sample was 2 µL.

Curves Calibration

Stock solutions of 1 mg/ml for each pesticide were prepared in methanol following by number of dilutions ranging from 0.01 to 10 µg/ml in methanol. The GC-ECD was standardized on the same day as samples were run. A mix standards solution was also prepared in methanol to reduce error. Standards were analyzed by injecting 2 µl of 3 standards and mix standard individually. Standard curve was plotted by area under peaks versus concentrations of fit by simple linear regression. Amount of each pesticide was calculated on base of slope of standard curve.

Recovery Studies

Recovery was done by analyzing pesticides free samples. Standard solution of chlorpyrifos, profenofos and triazophos were added at rate of 500 µg/kg prior to extraction. Extraction and cleanup was done with same method as describe previously. Precision and recovery was measured by analyzing samples in triplicate of each vegetable and found satisfactory for the analysis of pesticide residues.

Statistical Methods

The concentration of pesticides residues in samples were expressed in form of mean and standard deviation (SD). The mean and standard deviation was calculated by using Microsoft Excel 2010.

Conclusion

Samples contaminated with OPPs residues above MRL indicated that these commodities were unfit for human consumption. Almost half of the samples were contaminated with studied pesticides residues. This is alarming situation for end user and governing authorities. This study urges Food and Environmental Department to screen all food commodities for pesticides residues to have actual scenario of pesticides residues status in food chain of Pakistan. This will help in establishing data base regarding these residues in food and formulating border line on their use. Use of the pesticides or chemicals which are not dangerous for human health should be encouraged and research in this perspective should be conducted to assure safety of consumers.

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