Removal of Pesticide Residues from Okra Vegetable through Traditional Processing

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Abstract: Demand for vegetables in Pakistan is constantly increasing to feed growing population. Pakistan is the second largest producer of okra and in Sindh okra is produced throughout the year. Okra crop is attacked by variety of insect pests and commercial okra production relies heavily on the pesticides belonging to organochlorine, organophosphate, carbamate, pyrethroid and neo-nicotinoid groups for pest control. Moreover, growers do not observe safety interval for okra harvest. Hence the okra sold in Pakistani markets is highly contaminated with pesticide residues. Aim of this research study was to determine the extent of pesticide residue decontamination in okra vegetable through traditional processing.

Okra crop was sprayed with bifenthrin, profenofos and endosulfan, and different processing were applied on okra such as washing, detergent washing, sun-drying and cooking, etc. Bifenthrin, profenofos and endosulfan pesticide residues were extracted from okra by solvent partitioning and cleaned up through Florisil column using organic solvents for elusion as described by EPA and FDA procedures. Cleaned up residues were analyzed through GC-μECD.

The results revealed that endosulfan levels were reduced to MRL by detergent washing (from 2.01 ppm in unwashed samples to 1.03ppm). Profenofos residues (3.21ppm) were reduced to MRL (2.0ppm) by detergent washing and by combination of plain water washing and frying. Bifenthrin MRL is very low (0.04ppm) and only combination of detergent washing and frying reduced residues from 0.311 ppm to 0.042 ppm.

Keywords: Okra, Pesticides, Traditional processing.

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) belongs to family malvaceae. It is an important vegetable crop grown extensively in Pakistan. The control of numerous insect pests on this crop envisages use of different insecticides as a traditional and normal practices [1,2]. As a matter of fact in order to combat the insect pest problem, lot of pesticides are used by the vegetable growers. For better yield and quality, insecticides are repeatedly applied during the entire period of growth and sometimes even at the fruiting stage by the farmers/growers which has lead to 13-14 percent of total pesticides consumption on vegetables as against 2.6 percent of cropped area [3].

Pakistan is the 2nd largest consumer of pesticides among the south Asian countries. About 27 % of the total pesticides are used on fruits and vegetable crops [4]. The Pesticide business started in Pakistan in 1954 with the import of 254 metric tons of formulated product which increased to 20,648 metric tons in 1986-87 and 44,872 tons in 1998. Their use is increasing at the rate of 25% a year all over the globe [5].

Pesticides not only persist in the vegetables but also contaminate water and soil. It then enters the food chain, and exhibit its presence in human blood through consumption of foodstuffs and water. Besides, pesticides contribute to environmental pollution, biodiversity losses and deterioration of natural habitats [6]. The instances of pest recovery, development of resistance to pesticides, secondary pest outbreaks and destruction of non-target species have also been recorded and agriculture sector is being highlighted as the most important source of adverse effects [7].

Insecticides are also extensively used for controlling many agricultural pests. Indiscriminate use of insecticides/pesticides particularly at fruiting stage and non adoption of safe waiting period however, leads to accumulation of pesticide residues in consumable vegetables. Contamination of vegetables with pesticide residues has been reported by several researchers [8,9]. A number of pesticides that are commonly found in our food have been identified to adversely affect human health.

There is no pesticide monitoring system in the country which usually culminates in to excess of pesticides residues beyond the MRLs. However, in the developed countries where regular pesticide residues monitoring system is well established, pesticides

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ISSN: 1814-8085 / E-ISSN: 1927-5129/12

residues are not a major concern and only 1-2% vegetables sample may exceed MRLs.

The uses of insecticides/pesticides such as bifenthrin, endosulfan, and profenofos have increased in okra vegetable crop over the years. Therefore, their monitoring is very important. Keeping in view the above facts, the research study was designed to estimate the effect of traditional processing methods on the reduction of pesticide residues and to evaluate the level of pesticides residues (Emamecti benzoate, Imidacloprid, bifenthrin, endoulfan, profenofos) present in commercially produced okra. In addition, the study may develop/suggest effective methods through which pesticides can be reduced/removed or even decontaminated.

MATERIALS AND METHODS

Pesticide Spray on Okra

Okra was grown on University farm (area about half acre) through organic farming without pesticide spray to serve as control. Three separate plots of okra (area about half acre each) were sprayed with each pesticides and were harvested next day for determining the effect of various traditional processing techniques such as washing, detergent washing, drying either by sun-drying or thermal dehydration and cooking etc. on the level of removal/reduction in pesticide residue contents. The pesticides, endosulfan, bifenthrin and profenofos were sprayed at recommended dose [10] with Knapsack sprayer (Table **1**). After about 24 hours the okra was harvested and packed in polyethylene bags and brought to the laboratory of Institute of Food Sciences and Technology, Sindh Agriculture University, Tandojam for further processing.

Tabble 1: Recommended Dose of Pesticides Per Acre

Pesticides	Formulation	Active ingredient
Bifenthrin	250 ml /acre	25 ml/acre
Profenofos	800 ml/ acre	400ml/acre
Endosulfan	600 ml/ acre	210 ml/acre

Traditional Processing of Okra Samples

The okra samples were subjected to different traditional processing techniques such as unwashed unprocessed, unwashed sun-dried, un washed dehydrated, un washed fried, blanched, plain washed unprocessed, plain washed sun-dried, plain washed dehydrated, plain washed fried, detergent washed unprocessed, detergent washed sun-dried, detergent washed dehydrated, detergent washed. Samples were prepared for extraction of pesticides residues and to determine the effect of traditional processing on pesticide residues.

Chemicals

Pesticide standards of high purity (97.4%) were obtained from Bayer Crop Sciences, and Commercial pesticides were purchased from local market Hyderabad-Sindh, Pakistan. The solvents of HPLC grade were acetonitrile, n-hexane and ethyl acetate purchased from Merck Germany.

EXTRACTION OF PESTICIDE RESIDUAL ANALYSIS

Extraction of Endosulfan and Profenofos Residues

30ml ethyl acetate was added in 25g of samples and 10g of sodium sulfate and homogenized in blender for 10 minutes. The homogenate was filtered twice with Whatman No.1 filter paper. The filtrate was dried in Rotatory evaporator. n-hexane was added in dried filtrate and then it was further processed for cleanup. In case of frying, dried sample were dissolved in n-hexane and partitioned with 50ml of acetonitrile in the separating funnel. Oil was removed with n-hexane and the lower layer containing acetonitrile was saved. Acetonitrile portion was partitioned with 60ml n-hexane in separating funnel, then 10ml 10% NaCl solution added. The lower layer was discarded, and the upper layer of n-hexane was saved and then further processed for clean up.

Extraction of Bifenthrin Residues

30ml of n-hexane was added in 25g of samples and 10g of sodium sulfate and homogenized in blender for 10 minutes. The homogenate was filtered twice with Whatman No.1 filter paper and the filtrate was further processed for cleanup. In the case of frying, samples were dissolved in n-hexane and partitioned with 50ml of acetonitrile in separating funnel. Oil was removed with n-hexane and the lower layer containing acetonitrile was saved. Acetonitrile portion was partitioned with 60ml n-hexane in separating funnel, then 10ml 10% NaCl solution added. The lower layer was discarded, and the upper layer of n-hexane was saved and then further processed for clean up.

Table 2: GC Parameters for Determination of Residues

Endosulfan	Bifenthrin	Profenofos
Oven: 280 °C	Oven: 280°C	Oven: 220 °C
Injection port: 250 ◦C	Injection port: 250 ◦C	Injection port: 270 ◦C
Detector: 320 ◦C	Detector: 310◦C	Detector: 280 ◦C
Injection volume 2μl	Injection volume 2μl	Injection volume 2μl

CLEANUP OF PESTICIDE RESIDUES

Clean Up of Endosulfan, Profenofos and Bifenthrin Residues

Pesticide residues were cleaned up through Florisil column using n-hexane for elution. Cleaned up residues were analyzed through Gas Chromatograph coupled with micro-ECD.

Recovery Percentage

In order to ensure quality assurance information, before taking up analysis of test samples, the analytical method was standardized by processing spiked samples. Okra samples were taken from control plots where no insecticide had been sprayed. Samples were cut into small pieces of about 1–1.5 cm which were thoroughly mixed by tumbling. After quartering, 200–250g pieces were homogenized in a warring blender. Homogenized matrix (20 g), in three replicates was spiked with endosulfan, profenofos and bifenthrin separately at the fortification levels of 0.25, 0.50 and 0.75 ppm. Control samples were processed along with spiked ones. The processes of extraction, cleanup of pesticide residues were same as described above. Average per cent recoveries were 83.93 and 84.36 for endosulfan, 77.48 and 78.00 for profenofos and for bifenthrin it was 89.35 and 88.91. Recoveries were considered satisfactory for all above insecticides in Okra with the proposed methods. Retention times and peak areas of the studied pesticides in samples were comparable with the relative standards.

Stability of Standard and Working Solution

Standard stock solutions and working solutions were kept in freezer at -18^{o}C and were found to be stable for 6–8 months. Repeatability was also found to be quite satisfactory.

GC-μECD Determination of Endosulfan, Profenofos and Bifenthrin

It was carried out through following regimen and settings: Agilent 7890A gas chromatography, Injector autosampler 7683-B, Capillary column HP-5MS (30 m × 0.320 mm × 0.25μm), Detector μ-ECD, Gas liquid chromatography (GLC) operating parameters (Table **2**).

RESULTS

During the processing of okra such as the frying, sun drying and thermal dehydration weight loss occurred. During frying 57.22% of weight of okra was reduced due to the loss of water. The sun drying and thermal dehydration reduced the weight of okra by 65.8% and 80.80%, respectively as shown in Table **3**. The results of pesticides were shown by applying the weight loss effect.

Endosulfan residues up to 36.42% were reduced by plain washing. Sun-drying and drying through dehydrated chamber reduced at the level of 10.7% and 3.57% respectively. In fried sample endosulfan was reduced by 14.21%. Detergent washing was most effective and reduced residues by 48.9%. Detergent washing in combination with other techniques such as

Table 3: Weight Loss of the Samples Due to Loss of Water in Different Processes

Oil fried samples		Sun dried samples		Chamber dehydrated samples	
Before frying	50g	Before sun drying	50g	Before dehydration	50g
After frying	21.3g(±4.2)	After sun drying	17.1g(±5.3)	After dehydration	9.6g(±2.4)
Weight loss (%)	57.22	Weight loss (%)	65.8	Weight loss (%)	80.8

Table 4: Effect of Traditional Processing on Endosulfan Pesticide Residues

Treatment	Residues(ppm)(Mean±SD)	Reduction (%)
Unwashed unprocessed	2.01±0.12	0.00
Unwashed Sun-dried	1.79±0.11	10.70
Unwashed dehydrated	1.94±0.12	3.57
Unwashed fried	1.72±0.14	14.21
Plain washed unprocessed	1.28±0.10	36.42
Plain washed sun-dried	1.15±0.11	42.50
Plain washed dehydrated	1.21±0.12	39.60
Plain washed fried	1.15±0.13	42.71
Detergent washed unprocessed	1.03±0.09	48.90
Detergent washed sun-dried	1.01±0.09	49.64
Detergent washed dehydrated	1.04±0.08	48.21
Detergent washed fried	0.63±0.06	68.41
Blanching	1.09±0.09	45.70

MRL= 1 ppm.

frying, drying through dehydration chamber or sun-drying was more effective in reducing the residues (Table **4**).

Profenofos was reduced up to 25.13% by plain washing. Subsequent frying of plain washed okra reduced the residues up to 46.41% and the values were within MRLs. Detergent washing alone contributed 60.5% reduction and lowered the residues within MRLs and making okra fit for human consumption. All the detergent washed okra samples after further processing also had the residues within MRLs (Table **5**).

Bifenthrin, a pyrethroid insecticide, was reduced to the extent of 76.8% by sun-drying and 65.7% by

Table 5: Effect of Traditional Processing on Profenofos Pesticide Residues

Treatment	Residues (ppm) (Mean±SD)	Reduction (%)
Unwashed unprocessed	3.21±0.21	0.00
Unwashed Sun-dried	2.87±0.19	10.56
Unwashed dehydrated	2.99±0.14	6.85
Unwashed fried	2.24±0.15	30.10
Plain washed unprocessed	2.40±0.16	25.13
Plain washed sun-dried	2.22±0.15	30.80
Plain washed dehydrated	2.25±0.18	29.60
Plain washed fried	1.72±0.09	46.41
Detergent washed unprocessed	1.26±0.02	60.50
Detergent washed sun-dried	0.99±0.08	68.90
Detergent washed dehydrated	1.11±0.08	65.40
Detergent washed fried	0.41±0.05	86.95
Blanching	1.96±0.44	38.90

MRL=2 ppm.

Table 6: Effect of Traditional Processing on Bifenthrin Pesticide Residues

Treatment	Residues (ppm) (Mean±SD)	Reduction (%)
Unwashed unprocessed	0.311±0.095	0.00
Unwashed Sun-dried	0.072±0.073	76.8
Unwashed dehydrated	0.106±0.092	65.7
Unwashed fried	0.072±0.006	76.9
Plain washed unprocessed	0.275±0.081	11.45
Plain washed sun-dried	0.047±0.008	84.8
Plain washed dehydrated	0.072±0.051	76.9
Plain washed fried	0.070±0.009	77.3
Detergent washed unprocessed	0.233±0.054	24.7
Detergent washed sun-dried	0.042±0.009	86.6
Detergent washed dehydrated	0.056±0.008	81.9
Detergent washed fried	0.066±0.009	78.8
Blanching	0.198±0.071	36.2

MRL= 0.04 ppm.

dehydrated chamber. The plain washing reduced the residues by 11.45% and detergent washing reduced residue levels by 24.7%. Frying of sample reduced the pesticides by 76.9%. Moreover combination of detergent washing with sun-drying reduced bifenthrin level up to 86.6 and the residues were within MRLs (Table **6**).

DISCUSSION

Monitoring of pesticides is conducted globally to assess the environmental load of their residues. Current trend of increased pesticides use in the world as an alternative pest control is reflected in persistent organochlorines [6, 11]. Because of extensive use of pesticides, the presence of their toxic residues [12] have been reported in various environmental components/ commodities [8, 9,13, 14-17]. These pesticide residues find their way into the human body through food, water, and environment. Thus, analysis of pesticide residues in food and other environmental commodities like soil, water, fruits, vegetables and total diet have become essential requirement for consumers, producers, and food quality control authorities.

Endosulfan levels were reduced to MRL (0.1ppm) by detergent washing (from 2.01 ppm in unwashed samples to 1.03ppm). Profenofos residues (3.21ppm) were reduced to MRL (2.0ppm) by detergent washing and by combination of plain water washing and frying. Bifenfenthrin MRL is very low (0.04ppm) and only combination of detergent washing and frying reduced residues from 0.311 ppm to 0.042 ppm.

Washing decreased the residues of pesticides such as bifenthrin (11.4%), endosulfan (36.42%) and profenofos (25.13%). Important factor during the washing operation was the solubility of pesticides in tap water as well as in detergent water. Therefore, detergent washing increased the solubility and reduced the pesticides from its MRL value.

Blanching treatment reduced the endosulfan 45.7%, bifenthrin 36.2% and profenofos 38.90%. Blanching was more effective in eliminating pesticide residues as compared to tap water washing.

These operations such as washing, peeling, blanching and cooking play a role in the reduction of residues [18]. Each operation has a cumulative effect on the reduction of the pesticides present [19].

Thus, it is concluded from this study that fat soluble pesticides such as endosulfan, bifenthrin, profenofos were more affected by frying and less affected by washing because majority of pesticides were absorbed by the surface of okra due to its porous layer. The bifenthrin was more affected by sun because it is hydrolyzed in the presence of UV rays. In detergent washed sun-dried okra residues were further reduced to within MRLs.

Reduction percentage of pesticides residues in okra through traditional processing may help in reducing pesticide residues in marketed okra within MRLs and render it fit for human consumption.

ACKNOWLEDGEMENTS

This research study was funded by Pakistan Science Foundation, Islamabad. We also acknowledge the Director, National Centre of Excellence in Analytical Chemistry, Jamshoro and Mr. Tanveer Ahmed, Regional Manager, Ali Akbar Group (Pvt.) Ltd, Hyderabad for their assistance and helpful activity.

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Received on 28-01-2012 Accepted on 12-02-2012 Published on 17-02-2012

DOI: http://dx.doi.org/10.6000/1927-5129.2012.08.01.23