Effect of Household Processing on the Removal of Pesticide Residues in Okra Vegetable

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Abstract: The study has been designed to determine the extent of pesticide residues removal from okra through household processing. For this, okra crop was grown on university farm and application of pesticides were carried out at recommended dosage. After 24 hours, the okra was harvested, labeled and brought to the laboratory of Institute of Food Sciences and Technology, Sindh Agriculture University, Tandojam for their analyses and further processing such as washing, detergent washing, sun-drying and cooking, etc. being practiced at various households. Pesticide residues were extracted from okra by solvent partitioning and cleaned by C_{18} cartridges/activated charcoal by using acetonitrile for elution and then cleaned up residues were analyzed through HPLC-UV.

The analysis of data revealed that imidacloprid is highly effective against pests at low dosages and its residues in processed as well as unprocessed okra samples were within MRLs (0.5ppm). Imidacloprid residues 0.31 ppm in unwashed okra was reduced to 0.082 ppm by detergent washing (73% removal). Emamectin benzoate residues were high in unwashed okra (0.51 ppm as against MRLs of 0.2ppm), however, its residues were reduced to MRLs by detergent washing and subsequent processing by frying, thermal dehydration or sun-drying of detergent washed okra.

Keywords: House hold processing, Okra, Pesticides, HPLC.

INTRODUCTION

Vegetables are principal ingredient of our food having a high nutritional value. Vegetables such as okra, cauliflower, chilli, brinjal, bitter gourd and carrots etc. are grown for local consumption as well as for export purposes [1].

Okra (*Abelmoschus esculentus* L.) belongs to family malvaceae. It is an important vegetable crop produced extensively in Pakistan. It plays an important role in farmers economy as it gives better return over investment [2]. Literature revealed that okra crop is severely affected by insects and pests [3,4]. The control of numerous insect pests on this crop envisages use of different insecticides as a traditional and normal practice [5,6]. In order to combat the insect pest problem, lot of pesticides are being used by the vegetable growers. Besides, for better yield and quality, farmer apply pesticides belonging to organochlorine, organophosphate, carbamate, prethroid and neo-nicotinoid groups for the control of insects and pests. These 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% of total pesticides consumption on vegetables as against 2.6 percent of cropped area [7].

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The uses of insecticides such as emamectin benzoate and Imidacloprid, have increased in okra vegetable crop over the years. Therefore, their monitoring is very important to determine the extent of use and associated residues. In view the above facts, the research study therefore, was designed to estimate the effect of household processing methods on the removal of pesticide residues and to evaluate the level of pesticides residues present in commercially produced okra.

There is no formal or informal pesticide monitoring system in the country which has lead to excess of pesticides residues beyond the MRLs. However, in the developed countries where regular pesticide residues monitoring system is well established and pesticides residues are not a major concern and only 1-2% vegetables sample may exceed MRLs.

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. Two 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

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the level of removal/reduction in pesticide residue contents. The pesticides, imidacloprid and emamectin benzoate were sprayed at recommended dose 16 and 38ml/acre respectvely [8] with Knapsack sprayer. 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.

Household 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 Imidacloprid and Emamectin Benzoate Residues

30ml of acetonitrile 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 case of fried samples, the filtrate was partitioned with 50ml n-hexane in separating funnel. The upper layer of n-Hexane containing lipids was discarded, whereas lower layer containing acetonitrile and pesticide residues was subjected to cleanup.

CLEAN UP OF PESTICIDE RESIDUES

Clean Up of Imidacloprid and Emamectin Benzoate Residues

Extracts containing residues were cleaned up from interfering materials through activated charcoal. Charcoal was activated by heating in oven for about 3 hours at the temperature of 115^{o}C. 1g of activated charcoal was then added to each extract and the mixture was shaken for 20 minutes and then was vacuum filtered. The filtrate containing cleaned up residues were analyzed through HPLC.

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 imidacloprid and emamectin benzoate 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 78.5 and 80.1 for imidacloprid and 78.21 and 79.3 for emamectin benzoate. 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.

Derivatization and Determination of Emamectin Benzoate

Fluorescence derivatization was modified by the Method of Standard to Withhold Registration of emamectin benzoate samples. To the sample standard, 0.1 ml of 1-methylimidazole (99%) was added. The tubes were capped and vortex-mixed, and all samples, standards and the freshly prepared trifluoroacetic anhydride-acetonitrile (1:3) were placed in a cooling box at ice temperature for 10 min. After cooling, 0.3 ml of the trifluoroacetic anhydride–acetonitrile (1:3) was added to each sample and the standard tube. The tube was capped and allowed to stand for 10 min. The sample and standards were

Table 1: 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

diluted to 2 ml with acetonitrile in a volumetric flask and determined by liquid chromatography [12].

Determination of Pesticide Residues

HPLC Determination of Emamectin Benzoate

The LC system included a Hewlett-Packard 1100 liquid chromatograph with fluorescence detector operated at an excitation wavelength of 365 nm and emission wavelength of 470 nm. LC separations were performed with a 250 mm ×4.6 mm I.D. The flow-rate was 1.0 ml/min. Solvent-A was acetonitrile and solvent-B was water. The gradient elution conditions were initially A–B (80:20). The injection volume was 20 µl.

HPLC Determination of Imidacloprid

Separation was carried out on a Supelco LC-18 column (250mm× 4.6mm ID, 5µm) (Supelco Park, Bellefonte, USA). The mobile phase was acetonitrile +5mM ammonium acetate (20 + 80 by volume) at a flow-rate of 1.0ml/min and the detection wavelength was 270 nm. The injection volume was 20 µl.

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 reflected in Table **1**, by applying the weight loss effect.

Imidacloprid is an effective pesticides and it is applied at low dose. Hence its residues in non-processed and processed samples were within MRLs. Imidacloprid was reduced during plain washing and by detergent washing at a level of 27.69 and 73.23% respectively. Sun-dried and dried by dehydration chamber reduced the residues at the level of 18.9% and 8.9% respectively. Frying of okra showed no

Table 2: Effect of Household Processing on Imidacloprid Pesticide Residues

Treatment	Residues (ppm) (Mean±sd)	Reduction (%)
Unwashed unprocessed	0.31±0.051	0.00
Unwashed Sun-dried	0.25±0.061	18.90
Unwashed dehydrated	0.28±0.025	8.89
Unwashed fried	0.30±0.071	1.44
Plain washed unprocessed	0.22±0.061	27.69
Plain washed sun-dried	0.21±0.09	32.02
Plain washed dehydrated	0.228±0.06	26.38
Plain washed fried	0.226±0.04	26.9
Detergent washed unprocessed	0.082±0.007	73.23
Detergent washed sun-dried	0.088±0.008	71.39
Detergent washed dehydrated	0.082±0.0093	73.62
Detergent washed fried	0.0899±0.014	71.01
Blanching	0.158±0.041	48.95

MRL 0.5 ppm.

significant effect on pesticides residues of imidacloprid. All the samples of imidacloprid were less than its MRL value because it's low application of 80ml/acre (Table **2**).

The emamectin benzoate was reduced to the extent of 24.13% by plain washing. Drying by sun or in dehydrated chamber or frying alone did not effectively remove the residues (Table **3**). Detergent washing alone reduced the residues by 60.5% and the residue values were within MRLs. Subsequent processing of detergent washed okra samples had the pesticide levels within MRLs value and were fit for human consumption.

DISCUSSION

Imidacloprid is highly effective against pests at low dosages and its residues in processed as well as unprocessed okra samples were within MRL (0.5ppm). Imidacloprid residues 0.31 ppm in unwashed okra was reduced to 0.082 ppm by detergent washing (73% removal). Emamectin benzoate on the other hand, residues were high in unwashed okra (0.51 ppm as against MRL of 0.2ppm), however, its residues were reduced to MRLs by detergent washing and subsequent processing by frying, thermal dehydration or sun-drying of detergent washed okra. These results are in agreement with the findings of Amoah *et al*., [9].

Unwashed unprocessed okra contained maximum residues. The viable option is to reduce pesticide residue through traditional processing. The household processing decreased pesticides residues progressively. Washing with detergent removed residues adsorbed on okra surface which formed the major portions of polar pesticides such as imidacloprid and emamectin benzoate. Blanching treatment also reduced the water soluble pesticides such as imidacloprid and emamectin benzoate but frying of the vegetables did not show any significant effect on imidacloprid and emamectin benzoate residues because of high boiling point and insoluble in fat and oil.

Washing decreased the water soluble pesticides imidacloprid (27.69%), and emamectin benzoate (24%). 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 also reduced the imidacloprid 48.95% and emamectin benzoate 41.7%. Blanching was more effective in eliminating pesticide residues as compared to tap water washing.

All these operations such as washing, detergent washing, blanching and cooking/boiling play a role in the reduction of residues. These results were also supported by Randhawa *et al*., [10]. Each operation

Table 3: Effect of Household Processing on Emamectin Benzoate Pesticide Residues

Treatment	Residues(ppm) (Mean±sd)	Reduction (%)
Unwashed unprocessed	0.51±0.04	0.00
Unwashed Sun-dried	0.488±0.08	4.21
Unwashed dehydrated	0.511±0.06	-0.38
Unwashed fried	0.504±0.07	1.14
Plain washed unprocessed	0.386±0.05	24.13
Plain washed sun-dried	0.373±0.03	26.8
Plain washed dehydrated	0.389±0.04	23.75
Plain washed fried	0.356±0.03	30.26
Detergent washed unprocessed	0.201±0.02	60.50
Detergent washed sun-dried	0.197±0.02	61.30
Detergent washed dehydrated	0.205±0.02	59.77
Detergent washed fried	0.197±0.01	61.30
Blanching	0.297±0.07	41.70

MRL= 0.2 ppm.

has a cumulative effect on the reduction of the pesticides present [11]. Reduction percentage of pesticides residues in okra through household processing may help in reducing pesticide residues in marketed okra within MRLs and render it fit for human consumption. Similar results have also been reported by Baig *et al*., [2].

CONCLUSION

From these household processing, It is therefore concluded that water soluble contact pesticides residues such as Imidacloprid and Emamectin benzoate can successfully be removed from Okra by plain washing and/or by detergent washing. Vegetable consumers are advised to take vegetable after treatment of properly being washed and then fried for at least 10 minutes in oil.

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