Residue Removal of Pesticides from Brinjal Using Different Processing Methods

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Abstract: The present study was conducted on the residual removal of six pesticides (bifenthrin, profenofos, endosulfan, imidacloprid difenthiuron and emamectin benzoate) from brinjal by using various house hold processing methods such as washing, detergent washing, peeling, drying and cooking/frying etc. The data revealed that high amount of all pesticide residues (above MRLs) were present and unfit for human consumption in raw form. From water soluble pesticides diafenthiuron reduced more in blanching as compared with imidacloprid and emamectin benzoate. Frying reduced the residues more effectively, rendering it fit for human consumption. Washing followed by frying reduced the fat soluble residues >70% whereas water soluble pesticide residues >80%. It was concluded that each processing was found to be effective in reduction of pesticides residues.

Keywords: Brinjal, pesticides, extraction, cleanup, GC, HPLC.

INTRODUCTION

Brinjal (*Solanum melongena* L.) is an important vegetable crop in Pakistan. It is low in calories and fats, contains mostly water, some protein, fiber and carbohydrates. Brinjal production in Pakistan was noted at 8767 hectares with production rate of 85965 tones. Sindh Province contributed its growth on 1493 hectares with production of 6362 tones [1]. Brinjal is beneficial for diabetic and liver patients [2]. There are many varieties grown in Sindh, Pakistan. Brinjal (unripe) is consumed as vegetable in various ways and dried shoots are used as fuel in rural areas. Vegetables are important constituents of daily diet. These are more prone to insect pests i.e., jasssids, caterpillars, aphids, diamond moths, etc. In Pakistan, cauliflower, brinjal, okra and tomato, etc., are most commonly cultivated and consumed among the vegetables. But unfortunately are adeversly affected by the diseases and contamination by various pests' insects' rodents' birds etc.

Brinjal crop severely attacked at its fruiting stage due to fungal diseases and pest attacks i.e., jassids, fruit and shot borer, red spider mites, beetles, aphids and mealy bugs. Most of the damage is caused by fruit borer (70%) to the crop which is unfit for human consumption [3]. Mukhopadhyay and Mandal [4] mentioned that infestation of fruit borer in brinjal is specific to varieties. Kumar and Sadashiva [5] stated that brinjal shoot and fruit borer is a serious pest in which even a ready brinjal crop could collapse and 10-50% infestation of fruit borer is considered disastrous for the brinjal crop.

The chemicals (pesticides) are widely used during fruit and vegetable production to control attack of insects-pests. These find there ways inside the fruits and vegetables and become their component due to their direct application and remain inside at the time of harvest. There are many health, environment related hazards of pesticides have been documents by various researchers [6]. Many developed countries have recommended Maximum Residue Limit (MRL) based on the Acceptable Daily Intake (ADI) and Potential Daily Intake (PDI) of almost all pesticides for the safety of consumers [7], which should not be exceeded for a food item to be considered safe for consumption.

It is assumed that use of toxic pesticides on vegetables have raised the risk of intoxication of consumers along with diseases [8]. Farmers believe that for better yield the pesticide application is very important parameter to be conducted during crop production; by following this they tremendously apply the pesticides over crops even on fruiting. This repeated application of pesticides on crops particularly at fruiting stage and non adoption of safe waiting period leads to accumulation of pesticide residues in fruits and vegetables. Since some pesticides are water soluble and others are fat soluble, these pesticides when ingested even in minute quantities can results in the accumulation of pesticides inside the tissues of body and harm the consumer with its adverse effects [9].

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ISSN: 1814-8085 / E-ISSN: 1927-5129/14

Since pesticides use during cropping have now become an important step to prevent the attack of different pest related diseases, it is therefore mandatory that pesticide residues might be determined in vegetables before consumption. On the basis of this many developed and developing countries have established systematic monitoring programs for determination of pesticide residues from vegetables quantitatively as these residues (above the maximum tolerance limits (MRL)) if remained inside the fruits and vegetables at the time of harvest have believed to pose many hazardous effects to consumers nationally and globally [10]. According to the survey conducted by Karanath (2002) [11] that about 50-70% of the vegetables are contaminated with the residues of pesticides.

The present study was undertaken to determine the levels of pesticide residues present in brinjal and also the effects of various traditional processing methods on the removal of these residues so that contamination in vegetables due to application of pesticides can be removed or reduced before consumption.

MATERIALS AND METHODS

Pesticide Spray on Vegetables

Separate supervised pesticide application experiments were conducted on farmers' fields within 5-mile radius of SAU, Tandojam. Six pesticides (bifenthrin, endosulfan, profenofos, imidacloprid, diafenthiuron and emamectin benzoate) were sprayed with Knapsack sprayer, at recommended dosages given (Table **1**) on separate plot of brinjal.

Table 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
Imidacloprid	80 ml /acre	16ml/acre
Emamectin benzoate	200 ml/acre	38 ml/acre
Diafenthiuron	300 ml /acre	150 ml /acre

Brinjal was harvested next day after pesticide application as per routine practices of local farmers. Vegetable samples, were packed in properly labeled polythene bags and brought to the laboratory of Institute of Food Sciences and Technology, Sindh Agriculture University, Tandojam for further processing.

Processing Methods of Brinjal

The brinjal samples were subjected to different traditional processing techniques, such as unwashed unprocessed, detergent washed fried, detergent washed bhurta, plain washed samples were divided into two groups viz. peeled and unpeeled. Plain washed unpeeled samples were given treatments as plain washed unpeeled fried, plain washed unpeeled bhurta whereas, peeled brinjal samples were kept as plain washed and unwashed. Plain washed peeled samples were again washed with plain water and processed as plain washed peeled plain washed bhurta, plain washed peeled plain washed fried, plain washed peeled plain washed pickle and plain washed peeled plain washed blanched (Figure **1**). The samples were then packed in polyethylene bags with appropriate labeling and stored in deep freezer at -20°C till further processing.

Chemicals and Reagents

Extraction of Pesticide Residues

Endosulfan and Profenofos Residues

30ml ethyl acetate and 10g of sodium sulfate added in 25g of samples and homogenized in blender for 10 minutes. The homogenate was filtered twice with Whatman No.1 filter paper. The filtrate was dried in Rotary evaporator. The solvent n-hexane was added in dried filtrate and then further processed for cleanup. In case of fried samples, they 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 followed by addition of 10ml of 10% NaCl solution. The lower layer was discarded and the upper layer of n-hexane was saved and further processed for clean up.

Bifenthrin Residues

30ml of n-hexane and 10g of sodium sulfate added in 25g of samples 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 fried samples, they 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 of 10% NaCl solution was added. The lower

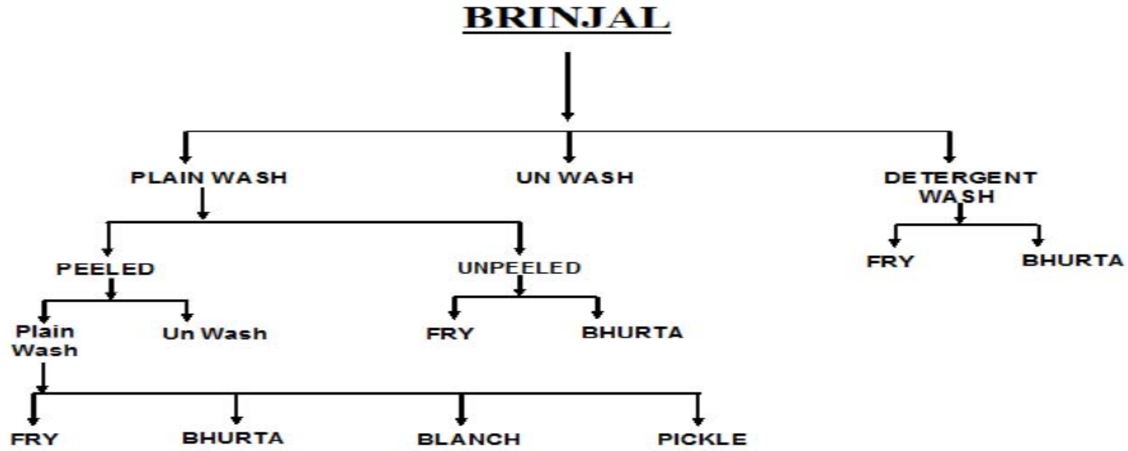

Figure 1: Processing methods of Brinjal.

layer was discarded and the upper layer of n-hexane was saved and then further processed for clean up.

Emamectin Benzoate and Imidacloprid Residues

30ml of acetonitrile and 10g of sodium sulfate was added in 25g of samples and homogenized in blender for 10 min. 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 oil was discarded, whereas lower layer containing acetonitrile and pesticide residues was saved and then further subjected to cleanup.

Diafenthiuron Residues

30ml acetone and 10g of sodium sulfate added in 25g of samples and homogenized in blender for 10 minutes. The homogenate was filtered twice with Whatman No.1 filter paper. The filtrate was dried in Rotary evaporator. The solvent acetonitrile was added in dried filtrate and then 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 oil was discarded, whereas lower layer containing acetonitrile and pesticide residues was saved and then further subjected to cleanup.

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 0.1ml 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 diluted to 2 ml with acetonitrile in a volumetric flask and determined by liquid chromatography [12].

Clean-Up of Pesticide Residues

Endosulfan, Profenofos and Bifenthrin Residues

Pesticide residues were cleaned up through Florisil column using n-hexane for elusion. The cleaned up residues were analyzed through Gas Chromato-graph coupled with micro-ECD.

Imidacloprid, Diafentiuron 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.

Determination of Pesticide Residues

GC-µECD Determination of Endosulfan, Profenofos and Bifenthrin Residues

For the determination of endosulfan, profenofos and bifenthrin GC-µECD were used which contained Agilent 7890A gas chromatograph, Injector auto-sampler 7683-B, Capillary column HP-5MS (30 m × 0.320 mm × 0.25µm), Detector µ-ECD. Following protocol was used for the analysis.

Table 2: Gas Chromatography Coupled with Micro ECD (GC-μECD) Parameters for Determination of gc Amenable Pesticides (Bifenthrin, Endosulfan and Profenofos Residues)

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

Table 3: HPLC Parameters for Determination of hplc Amenable Pesticides (Emamectin Benzoate, Diafenthiuron and Imidacloprid Residues)

Imidacloprid	Emamectin Benzoate	Diafenthiuron
Flow rate= 1.2ml/min	Flow rate = 1.2ml/min	Flow rate= 0.7ml/min
Ratio: Acetonitrile: Water (de-ionized) 35:65	Ratio: Acetonitrile: Water(de-ionized) 98:2	Ratio:Acetonitrile:Water(deionized) 85:15
Wavelength= 270nm	Wavelength=246nm	Wavelength= 250nm
Injection volume= 20μl	Injection volume=30 μl	Injection volume=20μl

HPLC Determination of Imidacloprid, Emamectin Benzoate and Diafanthiuron Residues

Separation was carried out on a Supelco LC-18 column (250mm× 4.6mm ID, 5μm) (Supelco Park, Bellefonte, USA). The mobile phase was acetonitrile and de-ionized water with UV (Ultraviolet) detector. Details of HPLC operation is given in Table **3**.

Recovery Percentage of Pesticide Residues

In order to ensure quality assurance information, before taking up analysis of test samples, the analytical method was standardized by processing spiked samples. Vegetable 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 (20g), in three replicates was spiked with emamectin benzoate, diafenthiuron, imidacloprid, endosulfan, profenofos and bifenthrin separately. Control samples were processed along with spiked ones. The processes of extraction, cleanup of pesticide residues were same as described above. Average percent recoveries were 85.21 for emamectin benzoate, 90.1 for diafenthiuron, 80.1 for imidacloprid, 84.36 for endosulfan, 78.00 for profenofos and for bifenthrin were 89.35. Recoveries were considered satisfactory for all above insecticides in brinjal 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 Solutions

Standard stock solutions and working solutions were kept in freezer at -18^{0}C and were found to be stable for 6–8 months. Repeatability was also found to be satisfactory.

Presenntation of Results

Residues were expressed as ppm. The residus of different pesticides detected in various vegetables samples were compared with Japanese MRL values.

RESULTS

Calibration Curves

The calibration curves of Bifenthrin, Profenofos, Endosulfan, Imidacloprid, Diafenthiuron and Emamectin Benzoate is presented in Fugures **2-7**. The calibration curves were plotted by the peak area of respective pesticides to concentration in ppm of pesticide standards which were used for quantification of pesticide residues from brinjal samples subjected to different processing technologies for the reduction of residues.

Effect of Traditional Processing on Pesticide Residues in Brinjal

Effect of Traditional Processing on the Weight Loss in Brinjal

The results revealed that during the processing of brinjal such as the frying weight loss occurred. During frying, the weight of brinjal was reduced to 13.01g due to the loss of water as shown in Table **4**. The results of pesticides are shown by applying the weight loss effect.

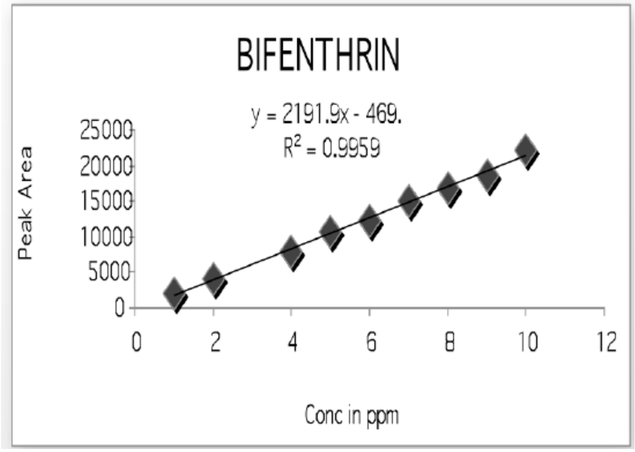

Figure 2: Caliberation curve of Bifenthrin residues.

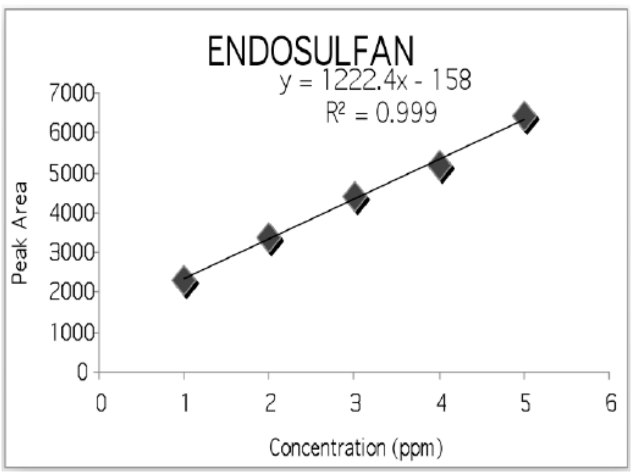

Figure 3: Caliberation curve of Endosulfan residues.

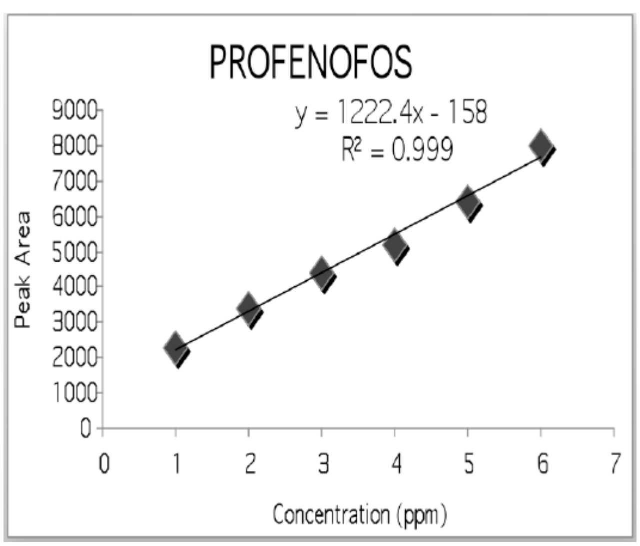

Figure 4: Caliberation curve of Profenofos residues.

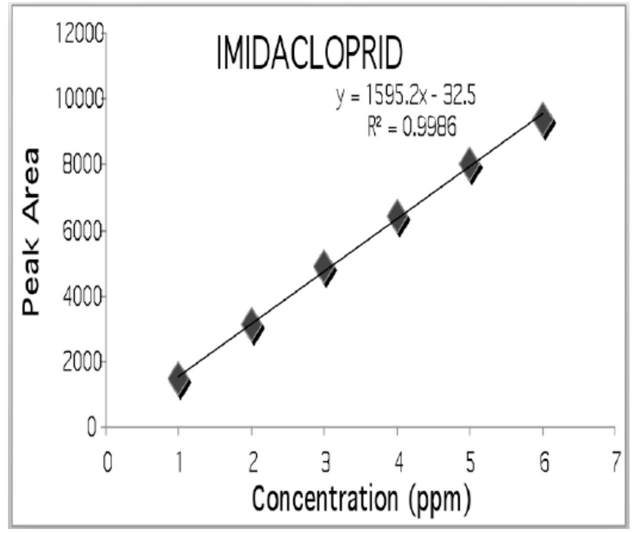

Figure 5: Caliberation curve of Emamectin Benzoate residues.

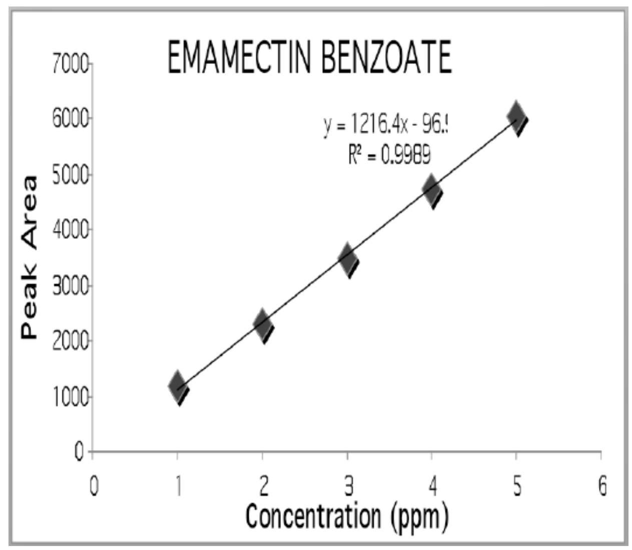

Figure 6: Caliberation curve of Imidacloprid residues.

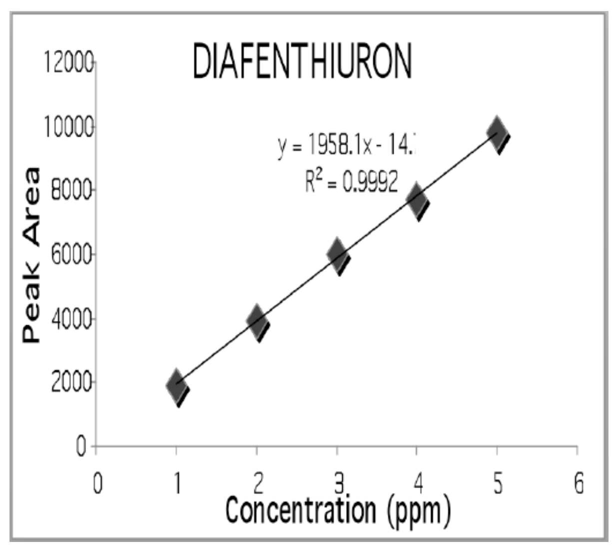

Figure 7: Caliberation curve of Diafenthiuron residues.

Table 4: Effect of Traditional Processing on the Weight Loss in Brinjal

Treatment	Weight (gm± se)	% of control	% weight loss	Concentration Factor
Before treatment	50.00	100	0	1
Oil fried	13.01±0.06	26.02	73.98	3.843198

Effect of Traditional Processing on the Reduction of Fat Soluble Residues in Brinjal

Bifenthrin, a pyrethroid insecticide, was reduced by peeled plain washed frying, blanching, bhurta and pickle up to 75.37, 29.91, 55.92 and 48.12% respectively. Frying and bhurta of detergent washed peeled sample on the other hand, reduced the pesticide residues up to 88.26 and 58.09% respectively. In profenofos peeling effectively removed the residues by 15.92%. The residues were further reduced by peeled plain washed frying 82.26%. Frying and bhurta of detergent washed peeled sample on the other hand, reduced the pesticide residues up to 90.32 and 66.37% respectively. Traditional processing reduced the endosulfan residues such as plain washing followed by frying, blanching, bhurta and pickle reduced the endosulfan residues up to 76.37, 34.94, 54.34 and 40.46% respectively (Figure **8**). The fried sample contained the residues within MRLs.

Effect of Traditional Processing on the Reduction of Fat Soluble Residues in Brinjal

Figure **9** showed that peeling effectively removed the imidacloprid residues by 13.67%. The residues were further reduced by peeled plain washed frying 80.58%. Frying and bhurta of detergent washed peeled sample on the other hand, reduced the pesticide residues up to 81.80 and 26.56% respectively. Diafenthiuron was reduced by peeled plain washed frying, blanching, bhurta and pickle up to 92.84, 37.50, 52.50 and 47.50% respectively. Frying and bhurta of detergent washed peeled sample on the other hand, reduced the pesticide residues up to 96.74 and 77.50% respectively. Frying and bhurta of detergent washed peeled sample on the other hand, reduced the pesticide residues up to 95.12 and 71.87% respectively of emamectin benzoate.

DISCUSSION

In this study concentration of pesticides from brinjal were analyzed through different processing methods. Pesticides are known to be present in vegetables due to extensive use of corresponding pesticides in interfiled cultivation.

In the present study it was observed that washing reduced the residue of fat soluble pesticides >15%, whereas water soluble pesticides by >19%. The results was according to [13] observations in which dissipation of bifenthrin residues in tomato was 0.107 and 0.234 mg/kg on 0 day after application and residues were reached below detectable level on 10 and 15th days after application. Kaur *et al*. (2011) [14] stated that preliminary deposit of cypermethrin was 0.6 and 1.095

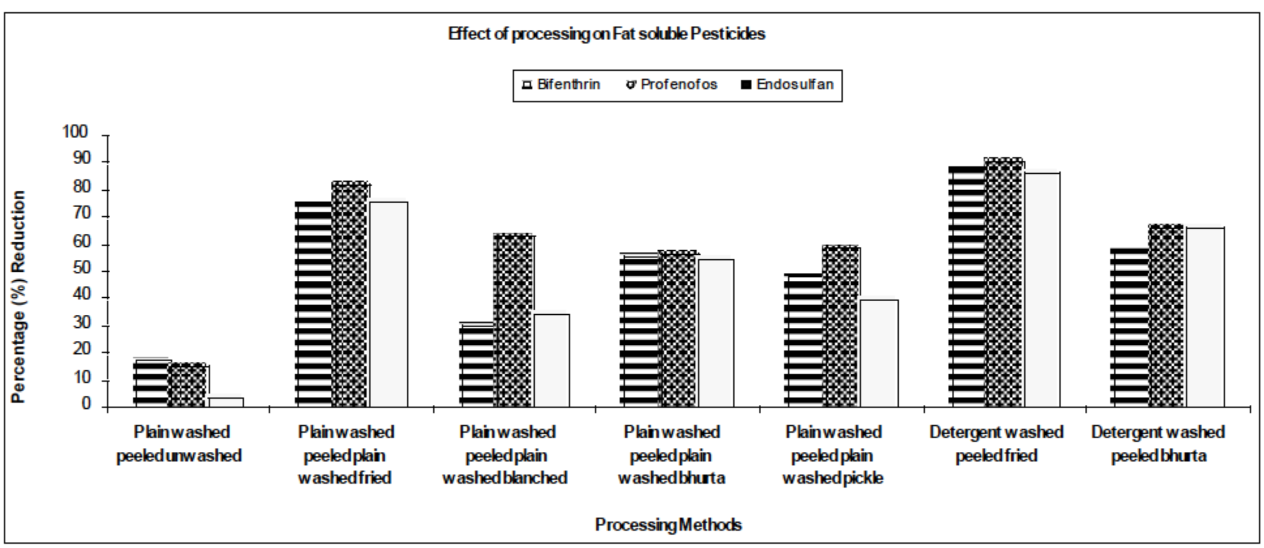

Figure 8: Effect of processing methods on reduction of fat soluble pesticides.

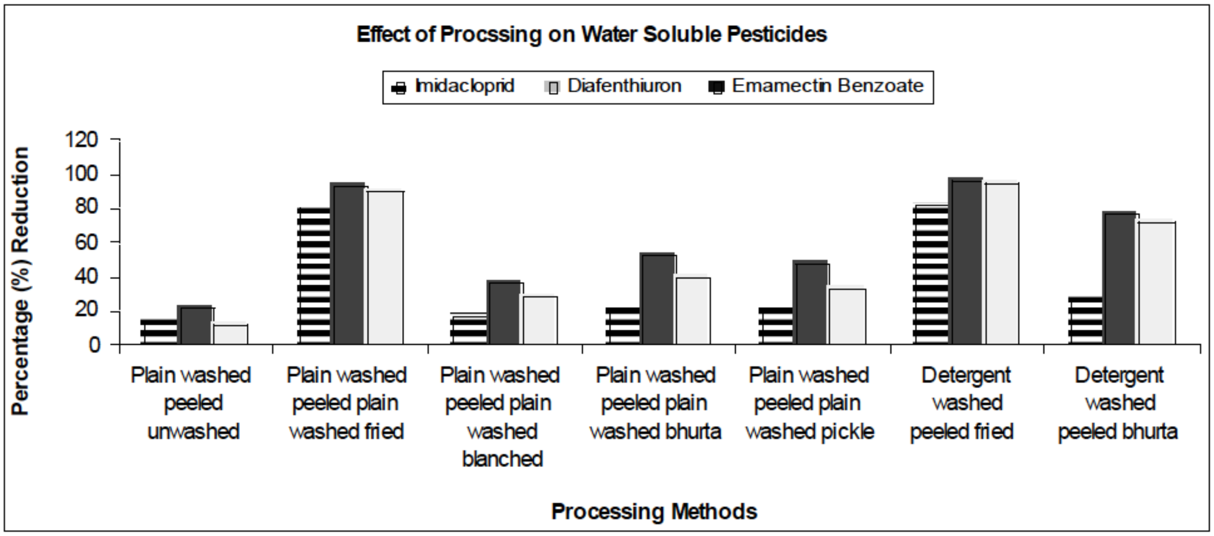

Figure 9: Effect of processing methods on reduction of water soluble pesticides.

mg kg-1 in brinjal which dropped to 0.1 and 0.189 mg kg-1, 1 day after treatment in case of single and double dose, showing percent dissipation of 95.0 and 94.06 on 10th day at single and double dose. Similar types of observation have been reported by [15].

Washing followed by frying reduced the fat soluble residues >:70% whereas water soluble residues >80%. However, detergent washing followed by frying reduced the fat soluble residues 88% whereas water soluble residues up to >90%. Present results are in agreement with earlier reports [16] in which 39.49 to 41.17% reduction of cypermethrin residues on Okra was observed, while in tap water washing along with cooking reduction was from 71.64 to 78.87%. Dislodging of cypermethrin (0.001%) residues after cooking of brinjal in boiled water reduced by 41.40 to 36.4% from 0 to 3 days samples [17].

Blanching of brinjal showed that profenofos of fat soluble pesticides reduced up to 65% then bifenthrin and endosulfan. However, from water soluble pesticides diafenthiuron reduced more in blanching as compared with imidacloprid and emamectin benzoate. Kaushik *et al.* (2009) [18] also supported the findings of the present study that blanching helps in reducing and hydrolyzing pesticide residues of non-persistent compounds. They further reported that non-polar residues are persistently accumulated in the waxy layers of the peel of vegetables and fruits. Sheikh *et al.* (2012 a,b) [19, 20] also reported that blanching treatments are more effective in the reduction of the pesticides residues i.e. endosulfan 45.7%, bifenthrin 36.2%, and profenofos 38.90% in okra.

CONCLUSION

It was concluded on the basis of the study that each pesticide contained high amount of residues in peel as well as edible. Frying reduced the residues more effectively, rendering it fit for human consumption. Traditional processing was found to be more effective in reduction of pesticides, it is therefore, recommended that vegetables should be processed traditionally before consumption and vegetable should not consumed in raw form.

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Received on 20-08-2014 Accepted on 10-09-2014 Published on 15-09-2014

http://dx.doi.org/10.6000/1927-5129.2014.10.57