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Effect of household processing on reduction of pesticide residues in bitter gourd (*Momordica charantia*)

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ABSTRACT

Gourds include a number of popular vegetables such as ridged gourd (ribbed goured), sponge gourd (*L. cylindrica*), bitter guord, snake gourd, ash or white gourd, and bottle gourd. Bitter gourd or karela, also known as balsam pear (*Momordica charantia*), is cultivated all over India for its bitter immature ridged fruits. It is believed to have originated in the tropical regions of the old world. India is the secondary centre of origin of this crop. It is widely cultivated as a vegetable crop in China, India, Malaysia and tropical Africa. Bitter gourd is very nutritious vegetable having high therapeutic value. Traditionally bitter gourd is eaten in the form of boiled and cooked as a subji and therefore raw, washed, boiled and cooked samples were selected for the pesticide residual analysis. The effects of household processing on pesticide residues were also studied. Literature reveals that vegetables may contain pesticide residues above the prescribed maximum residue levels (MRL), which may pose health hazard to the consumers. Analysis of eggplant for pesticidal contamination was carried out on Gas Chromatograph-Electron Capture and TID Detector with capillary columns. The study revealed that bitter gourd was found contaminated maximum with parathion and minimum with permethrin in the range of 3.45 and 0.30-0.40 $\mu g g^{-1}$, respectively. It was found that washing and cooking process minimized the pesticide residues of nine pesticides in the range of 1.74-64.78 and 38.40-90.15 per cent, respectively. The percentage reductions in the present study are supported by both early and most recent publications.

Key Words : Bitter gourd, Processing OC and OP pesticide reduction

INTRODUCTION

Vegetables are essential components of our diet due to their nutritional value. Fruits, nuts and vegetables play a significant role in human nutrition, especially as sources of vitamins (C, A, B6, thiamine, niacin, E), minerals, and dietary fiber (Quebedeaux *et al.*, 1988 and 1990; Wargovich, 2000). During the last two decades considerable emphasis has been laid on production of these crops in our country and vegetable exports have been stepped up (Karanth *et al.*, 1982). However, the development of the export market is hindered by concerns about chemical residues and inadequate monitoring. Pesticides are widely used to ensure high crop yields. They are used during production and post-harvest treatment of agricultural commodities (Levitt *et al.*, 2010). However, increased use of chemical

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pesticides has resulted in contamination of the environment and also caused many associated long-term effects on human health (Bhanti, 2007). The presence of pesticide residues in food commodities has always been a matter of serious concern. The problem is especially serious when these commodities are consumed (Solecki *et al.,* 2005). Contamination of vegetables with pesticide residues has been reported by many researchers (Holland *et al.,* 1994, Karanth *et al.*, 1982, Kilgore and Windham, 1970). Gourds include a number of popular vegetables such as ridged gourd (ribbed gourd), sponge gourd (*L. cylindrica*), bitter guord, snake gourd, ash or white gourd, and bottle gourd. Bitter gourd or karela, also known as balsam pear (*Momordica charantia*), is cultivated all over India for its bitter immature ridged fruits. It is an annual plant of slender climbing or trailing habit. The fruits are generally 10 -20 cm long, tapering at the ends and covered with blunt tubercles. They are green when unripe turning to an orange yellow color when ripe. Bitter gourd is believed to have originated in the tropical regions of the old world. India is the secondary centre of origin of this crop. It is widely cultivated as a vegetable crop in China, India, Malaysia and tropical Africa. Bitter gourd is very nutritious vegetable having high therapeutic value. It is a good vegetable for those suffering from diabetes. The young fruits are cooked and eaten; they are often steeped in salt water after peeling and before cooking to remove the bitter taste. Traditionally bitter gourd is eaten in the form of boiled and cooked as a subji and therefore raw, washed, boiled and cooked samples were selected for the pesticide residual analysis. The effects of household processing on pesticide residues were also studied.

METHODOLOGY

Chemicals :

Reagents:

Standard pesticides which were >98% pure were procured from RFCL, Delhi, India. HPLC grade hexane, acetone and ethyl acetate, and AR grade anhydrous sodium sulphate, sodium chloride, Florisil, Activated charcoal, Silica gel for column chromatography were procured from RFCL, Delhi, India.

Standard materials:

Standard pesticides which were >98% pure were procured from RFCL, Delhi, India. The standard stock solutions (100 ppm) were prepared in ethyl acetate and stored at -4^{0}C. Working standard mixtures of six pesticides in ethyl acetate, containing 10 µg/ml of each pesticide, were used for spiking the samples and preparing calibration standards.

Instruments :

(a) Blender-Boss Appliances, Daman, India

(b) Centrifuge-Kumar Industries, Bombay, India

(c) Mechanical shaker -Modern Industrial corporation, Bombay, India

(d) Rotary evaporator -Jain Scientific, India

(e) GC- Thermofisher 1000 GC equipped with capillary columns using ^{63}Ni electron capture detector (ECD) and TID.

(f) Capillary column- 1. SPB-5 of 5% diphenyl/ 95% dimethyl fused silica capillary column (30 m×0.32 mm ID, 0.25 ìm film thickness) 2. HP-1 of methyl silicone (10 m×0.53 mm ID, 2.65 µm film thickness).

Instrument conditions :

For OC : Temperatures (^{0}C):150 (5 min) → 8 ^{o}c min^{-1} → 190 (2 min) → 15 ^{o}c min^{-1} 280°C (10 min); injection port: 280 ^{o}C; detector: 300 ^{o}C; carrier gas: (N_2), flow rate 60 ml min^{-1}, 2 ml through column and

split ratio 1:10. Carrier gas, N_2, flow rate 60 ml min^{-1}, 2 ml through column.

For OP : Temperatures(^{0}C): Oven: 100 (1 min) $\rightarrow$ 10 ^{o}c min^{-1} $\rightarrow$ 200 ^{o}C (0 min) $\rightarrow$ 20 c min^{-1} $\rightarrow$ 260^0 C (3 min); injector port, 250 ^{o}C , detector, 275 ^{o}C, carrier gas N_2 18 ml min^{-1}, H_2, 1.5 ml min^{-1} and zero air 130 ml min^{-1}.

Sampling :

A total of 45 samples of various vegetables were commercially purchased from the local market of Rajkot city, Gujarat, India, during October, 2010 and February, 2011 and served as the blank or spiked sample. All the samples were extracted fresh. The unit was generally more than 250 g (Codex Alimentarius, 2000). For the analysis, only the edible portions were included, whereas bruised or rotten parts were removed.

Processing vegetables:

Vegetables like Bringal and bitter gourd samples, after washing, were hand peeled into slices with a stainless steel peeling knife and cooked. Spinach, cauliflower, cabbage tomato, okra were washed, sliced into a suitable size and cooked. Vegetable samples (raw) were dry, cleaned to remove soil contamination with a disposable paper towel and blended to mace a homogeneous sample for pesticide analysis.

Washing :

Vegetables were washed by placing in a plastic colander and rinsed under normal tap water (25-30^{0}C) for 30 second (Krol *et al.*, 2000; Thanki *et al.*, 2012).with gentle rotation by hands and blotted dry with a paper towel. These samples were divided into two portions, of which one was analyzed as such after homogenizing in blender and other was further boiled and cooked.

Boling:

Sliced vegetables were boiled by placing 75 ml of water in saucepan. Vegetable (50g) was added immediately to boil for 5-10 min / boiled still softness was subjected to pesticide analysis.

Cooking :

Sliced vegetables were cooked (Kilgore *et al.*, 1970) by placing 15 ml of water in saucepan. Vegetable (50g) was added immediately to cook for 10-12 min was subjected to pesticide analysis. Washed, boiled and cooked samples were processed in a similar manner as of unprocessed samples

Extraction :

Commercially purchased cabbage served as the blank or spiked sample. All the samples were extracted fresh. Each vegetable was chopped into small pieces and after quartering, a representative sample (50g) was macerated with 5-10g anhydrous sodium sulphate in Warring blender to make a fine paste. The macerated sample was extracted with 100ml acetone on mechanical shaker for 1 h by using the method of Kumari *et al.* (2001). Extract was filtered, concentrated up to 40ml and subjected to liquid-liquid partitioning with ethyl acetate (50, 30, 20 ml) after diluting 4-5 times with 10% aqueous NaCl solution. Concentrated the organic phase up to 10ml on rotary evaporator and divide it into two equal parts. One part was kept for OC and second for OP.

Clean-up :

For OC, clean-up was carried out by using column chromatography. Column (60cm × 22mm) was packed with, Florisil and activated charcoal (5:1 w/w) in between the two layers of anhydrous sodium sulphate. Extract was eluted with 125ml mixture of ethyl acetate: hexane (3:7 v/v). Eluate was concentrated

to 2ml for residue analysis.

Residues of OP were also cleaned by adopting column chromatographic technique. Column was packed with silica gel and activated charcoal (5:1 w/w) in between the layers of anhydrous sodium sulphate. Extract was eluted with 125ml mixture of acetone: hexane (3:7 v/v). After concentrating the eluate on rotary evaporator, final volume was made to 2ml for analysis by gas liquid chromatography (GC).

Quantization :

An external method was employed in the determination of the quantities of residues in the sample extracts. A standard mixture of known concentration of pesticide was run and the response of the detector for each compound ascertained. The area of the corresponding peak in the sample was compared with that of the standard. All analyses were carried out in triplicates and the mean concentrations computed accordingly.

Recovery rate and limit of detection:

Cabbage samples were fortifies at 0.01, 0.02 and 0.1 mg/kg by adding 5.0 mL of a mixed standard solution. Recovery and precision (expressed as relative standard deviation) were calculated for three replicate samples. Per cent recoveries in spiked samples ranged 87.3% -104.0 % (Zawiyah *et al*., 2007). Accordingly, the sample analysis data were corrected for these recoveries. Detection limit(s) of the method were also assessed based on the lowest concentrations of the residues in each of the matrices that could be reproducibly measured at the operating conditions of the GC; which were 0.001 mg/kg. Blank analyses were also carried in order to check any interfering species in the reagents.

Estimation:

The cleaned extracts were analyzed on Thermofisher 1000 GC equipped with capillary columns using ^{63}Ni electron capture detector (ECD) and TID. Operating conditions were as per details: For OC: Detector : ECD (^{63}Ni), column: SPB-5 of 5% diphenyl/ 95% dimethyl fused silica capillary column (30 m×0.32 mm ID, 0.25 μm film thickness) with split system. Temperatures (^{0}C):150 (5 min) $\rightarrow$ 8^{0}C min^{-1} $\rightarrow$ 190 (2 min) $\rightarrow$ 15^{0}C min^{-1} 280^{0}C (10 min); injection port: 280 ^{0}C; detector: 300 ^{0}C; carrier gas: (N_2), flow rate 60 ml min^{-1}, 2 ml through column and split ratio 1:10. Carrier gas, N_2, flow rate 60 ml min^{-1}, 2 ml through column.

RESULTS AND DISCUSSION

In the analyzed samples, the detected pesticides comprised of, phorate, parathion, chlorpyriphos, pendamethalin, carbaryl, endosulphan-II, captafol, permethrin and cypermethrin. The study revealed that bitter gourd was found contaminated maximum with parathion and minimum with permethrin in the range of 3.45 and 0.30-0.40 μgg^{-1}, respectively.

Although all the samples were found contaminated with OC insecticides but none of the samples contained residues of any of these insecticides above maximum residue limits (MRL) fixed by Prevention of Food Adulteration Act (PFA) 1954 and FAO/WHO (1996). As many organohalogen pesticides like BHC and DDT have been banned with effect from April 1993, In India, but they have remained in the environment where they continue to be incorporated into plant biomass. In India, practically, DDT has not been phased out completely because it is still used to control the mosquito in public health programmes from where it could enter the agricultural soils and water systems and possibly find its way into crops. Presence of endosulfan in the present study is due to use of endosulfan in almost every crop in Gujarat, India among the OC pesticides after banning of use of DDT and HCH in 1993. Residues of phorate (2.45-2.66 μgg^{-1}), chlorpyriphos (0.41-0.48 μgg^{-1}), pendamethalin (2.0-2.30 μgg^{-1}),

carbaryl (0.57-0.65μgg^{-1}) endosulphan-II (0.99-1.40 μgg^{-1}), captafol (1.07-1.30 μgg^{-1}) and cypermethrin (0.43-0.50 μgg^{-1}) were detected in bitter gourd. The results obtained from the present study are consistent with an earlier study that show residues of these pesticides are present in different vegetables (Madan *et al.*, 1996; Kumari *et al.*, 2002 and 2003; Deka *et al.*, 2005, Joshi *et al.*, 2011 and 2012).

Effects of household processing :

Among household processes washing process reduced the pesticide residues by 9.94-78.89 percent. Maximum reduction of residue was observed in case of captafol and pendamethalin where the residues decreased to the extent of 78.89 and 57.67 per cent by washing process respectively. In the present study washing was found effective in the decontamination of pesticide residues as it depends on a number of factors like, location and age of residues, water solubility, temperature and type of washing solution. In earlier studies also, effect of these factors were observed in different vegetables by various researchers (Madan *et al.*, 1996; Kumari *et al.*, 2002 and 2003; Deka *et al.*, 2005, Joshi *et al.*, 2011 and 2012). Washing found comparatively less effective in reducing the residues of permethrin (18.86), carbaryal (17.0), and parathion (9.94). Boiling was observed to be effective in reducing the residues. By this process, reduction of residues of nine pesticides was observed in the range of 16.66-85.47 per cent. Maximum reduction was observed in the case of captafol, pendamethalin and phorate where the residues decreased to the extent of 85.47, 84.66 and 62.02 per cent, respectively. Cooking was observed to be more effective in reducing the residues. By this process, reduction of residues of twelve pesticides was observed in the range of 22.39-94.64 per cent. The great variation in reduction of residues by boiling/cooking was observed which may be attributed to the rates of degradation and volatilization of residues as the concentration of residues increases by heat involved in boiling/cooking. Maximum reduction was observed in the case of captafol, pendamethalin and

Table 1 : Effect of processing on pesticide residues (μg g^{-1}) in bitter gourd

Sr. No	Name of pesticide	Raw (Mean)	Washing (Mean) [% Reduction]	Boiling (Mean) [% Reduction]	Cooking (Mean) [% Reduction]
1	Phorate	2.45-2.66	1.50-1.63	0.84-1.09	0.73-0.84
		(2.57)	(1.574)[38.75]	(0.976) 62.02]	(0.774) (69.88]
2	Parathion	3.45-4.26	3.01-3.80	2.8-3.71	2.54-3.20
		(3.922)	(3.532) [9.94]	(3.288) [16.16]	(3.044) [22.39]
3	Chlorpyriphos	0.41-0.48	0.30-0.38	0.15-0.34	0.11-0.16
		(0.438)	(0.338) [22.83]	(0.20) [54.33]	(0.128) [70.78]
4	Pendamethalin	2.00-2.30	0.80-1.20	0.27-0.40	0.21-0.30
		(2.138)	(0.916) [57.16]	(0.328) [84.66]	(0.258) [87.93]
5	Carbaryl	0.57-0.65	0.47-0.54	0.24-0.50	0.07-0.12
		(0.60)	(0.498) [17.00]	(0.306) [49.00]	(0.092) [84.67]
6	Endosulphan-II	0.99-1.40	0.65-0.70	0.59-0.62	0.52-0.57
		(1.138)	(0.676) [41.60]	(0.602) [47.10]	(0.542) [52.37]
7	Captafol	1.07-1.30	0.21-0.28	0.14-0.21	0.03-0.10
		(1.156)	(0.244) [78.89]	(0.168) [85.47]	(0.062) [94.64]
8	Permethrin	0.30-0.40	0.25-0.32	0.12-0.31	0.11-0.18
		(0.35)	(0.284) [18.86]	(0.19) [45.71]	(0.138) [68.57]
9	Cypermethrin	0.43-0.50	0.30-0.37	0.20-0.30	0.18-0.24
		(0.464)	(0.33) [28.88]	(0.244) [47.41]	(0.204) [56.03]

carbaryl where the residues decreased to the extent of 94.64, 87.93 and 84.67 per cent, respectively. Holland *et al*. (1994) reported appreciably reduction in pesticide residues in different commodities by using different processing methods. Hence, the present results are in consistent with the earlier results.

Conclusion :

It can be concluded that processing substantially lowers the residues of pesticides in bitter gourd. It was found that washing and cooking process minimized the pesticide residues of nine pesticides in the range of 1.74-64.78 and 38.40-90.15 per cent, respectively. The percentage reductions in the present study are supported by both early and most recent publications. These reductions are extremely important in evaluating the risk associated with ingestion of pesticide residues, especially in vegetables, which are eaten by almost all income groups' people. The present study showed that cooking was found more effective than washing.

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