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Effect of washing and boiling on pesticide residue reduction of diafenthiuron in Brinjal and cabbage

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Abstract
Supervised field trials were conducted in Ludhiana, Punjab for residue studies of diafenthiuron on brinjal and cabbage. Three spray applications of diafenthiuron at were made 10 day's interval @ 300 and 600 g i.e. ha^{-1}. The brinjal and cabbage samples were collected from field on 0 (2 hours) and 1 day after the second and third application for conducting decontamination studies. Both processed and unprocessed samples were analysed for diafenthiuron residues. The samples were made using Quenchers technique, quantified by using high performance liquid chromatography (HPLC) with photo diode array (PDA)detector and confirmed by LC-MS. Various household processing like washing removed 80.76 - 100% residue from brinjal and 66.66% from cabbage, while boiling of brinjal and cabbage substantially reduced diafenthiuron residues upto 100%. Both processing methods reduced the residues of diafenthiuron to a level of less than 1 mg kg^{-1} fixed by FSSAI.

Keywords: Boiling cooking cabbage brinjal diafenthiuron residues decontamination washing

1. Introduction

Vegetables are one of the most important component of human diet. They act as major sources of minerals and vitamins needed for human health (Gupta *et al* 2005) [1]. Cabbage (*Brassica oleracea var. capitata*.) and brinjal (*Solanum melongena* L.) are two major vegetable crops in India. In India, the area under brinjal and cabbage were 668720 and 406860 hectares having a production of 12399.90 and 8970.53 thousand MT respectively (Anonymous 2016-2017a) [2]. In Punjab, the area under brinjal and cabbage were 4260 and 5790 hectares with a total production of 92.39 and 101.31 thousand MT respectively (Anonymous 2016-2017b) [3]. In addition to various abiotic stresses, brinjal is subjected to severe and extensive damages by different insect pests. Among all the pests, shoot and fruit borer, *Leucinodes orbonalis* Guenee is the most serious pest causing yield loss upto 60-70% to the crop (Roy *et al* 2016) [4]. Among the sap feeders jassid, *Amrasca biguttula biguttula* Ishida, aphids *Aphis gossypii* (Glover) and whitefly *Bemisia tabaci* (Gennadius) are deadly pests. Insect pests are important constraints in cabbage production as they cause severe economic loss. Among those, Diamond back moth and tobacco caterpillar cause more economic damage and farmers are using high doses of new insecticides for the pest control

Vegetables are contaminated with residues and various pesticides have been already reported by several scientists (Kumari *et al* 2003) [5]. Many of the vegetables contain the pesticide residue levels exceeding maximum residue limit MRL (Taneja, 2005) [6] and May imparts various health problems to the consumers (Flliion *et al* 2000; Mukherjee and Gopal, 2003) [7, 8]. Repeated use of conventional insecticides providing adequate pest control in higher doses can cause adverse effects on the environment and health. Several hazards and risks are associated by the residues of pesticides left on the crop as well as soil. Therefore, the insecticide recommendation of an insecticide/pesticide should be such that requires that it can provide an effective pest control as well as residues on the commodities should be toxicologically acceptable.

Food processors and scientists are interested in studying the effect of household/commercial processing on removal of pesticide residues from fruits and vegetables. Several researchers studied the impacts of common processing methods on different kinds of produce and observed that residues were considerably reduced due to various treatments. Various processing methods like peeling, washing, blanching, cooking, concentrating can greatly Reduce residue level in food and then reduce impacts on human health(Abou-arab 1999; Byrne and Pinkerton 2004; Soliman 2001; Zohair 2001) [9, 10, 11, 12].

Since, literature related to studies on the effects of household processing on the reduction of diafenthiuron residues are not available, the current studies were planned to study the effect of processing like washing, boiling and removal of outer wrapper leaves on the reduction of diafenthiuron residues on brinjal and cabbage.

2. Materials and methods

2.1. Reagents and Chemicals

The technical grade analytical standards of diafenthiuron (purity 98%) was obtained from M/s Sigma Aldrich, India. The commercial formulation (Polo 50% WP) was supplied by M/s Syngenta India Pvt. Ltd., Pune, India. Analysis of the formulation showed only the presence of diafenthiuron without any of its metabolic product.

Polar solvents like acetonitrile (HPLC grade) and water (HPLC grade) were procured from Sisco Research Laboratories, Maharashtra. Sodium chloride (ASC reagent grade C99.9%; NaCl) was also obtained from Merck, Darmstadt, Germany. Sodium sulfate (Na2SO4) anhydrous (AR grade) was form's D. Fine Chemicals, Mumbai. Analytical grade activated anhydrous MgSO4 was also obtained from Merck, Darmstadt, Germany. Primary Secondary Amine (PSA) Sorbent and activated graphitized carbon black (GCB, 400mesh) were obtained from Sigma-Aldrich, Mumbai, India. Before use, all common solvents were redistilled in all-glass apparatus. For testing the suitability of the solvents and other chemicals, reagent blanks were run before actual analysis.

2.2. Standard solution preparation

A standard diafenthiuron stock solution (1 mg/mL) was prepared using HPLC grade acetonitrile. Different concentrations of standard solutions (1.00, 0.50, 0.25, 0.1 and 0.05 µg/mL) required for constructing a calibration curve were prepared from stock solution by doing serial dilution with acetonitrile of HPLC grade. All standard solutions were stored at 4^{o} C before use.

2.3. Field Experiments

Cabbage (var. Early drumhead) and brinjal (var. Punjab Neelam) were raised at Entomological Research Farm, Punjab Agricultural University, Ludhiana following Good Agronomic Practices (Anonymous 2017a) [13] in a plot having size of 50 m^2. There were three treatments i.e. control, single dose and double dose with three replication for each and followed randomized block design (RBD).The first application of diafenthiuron (Polo 50% WP) @ 300 and 600 g i.e. ha^{-1} was made at head formation stage in case of cabbage and fifty percent flower initiation stage in case of brinjal followed by second and third applications at 10 days interval. Insecticide was sprayed as foliar application with the help of ASPEE Knapsack sprayer.

2.4. Sampling

About 1 kg fruits/heads of marketable size were randomly collected 0 (1 h) and 1 days after the third insecticide spray. The brinjal fruits and cabbage heads were taken from each plot separately and carried to laboratory in plastic bags for processing.

2.5. Processing

In treatment one (T_1), each replicated brinjal fruits and cabbage heads, samples of 100 g each was washed for 2-under running tap water for 2–3 min. In the second treatment (T_2), the heads/ fruits were cooked in 500 mL boiling water for 2 mins. In the third treatment (T_3), the outer wrapper leaves of cabbage were removed and then cut into small pieces.

2.6. Extraction and Clean-up

The brinjal and cabbage samples were prepared by Quenchers technique for the determination of diafenthiuron residues. A sub sample of 15 g of brinjal fruits/cabbage heads were weighed into a 50 mL centrifuge tube and mixed with 15 mL acetonitrile. Anhydrous sodium chloride 5 ± 0.1 g was added to the centrifuge tube and shaken vigorously on rotospin (Tarson®) for 10 min at 50 rpm. The contents were centrifuged for 5 min at 2400 rpm. An aliquot of 10 mL acetonitrile was transferred \to a tube containing 10 ± 0.1 g sodium sulfate and shaken well to absorb moisture from the sample if any. An aliquot of 4 mL acetonitrile was taken in a tube containing 0.10 ± 0.01 g PSA sorbent and 0.60 ± 0.01 g anhydrous $MgSO_4$ and the content was thoroughly mixed on vortex spinix (Tarson®) to cleanup by dispersive solid phase extraction (DSPE). Again the contents were centrifuged for 3 min at 2400 rpm and a 2 mL aliquot of acetonitrile extract was collected for residue analysis.

2.7. Determination of Diafenthiuron residues

The quantification of diafenthiuron residues was done by using high performance liquid chromatography (HPLC). The HPLC (Model DGU-2045) equipped with reverse phase (RP) C_{18} column and photo diode array (PDA) detector, dual pump was supplied by M/S Shimadzu Corporation, Kyoto, Japan. The HPLC column, a Luna 5µm C_{18} column (250 x 4.6 mm size, 5.20±0.30 µm particle size, 2.20±0.30 (90%/10%) particle distribution, 95±15 A° pore diameter, 430±40 m^2 g^{-1} surface area, < 55.0 ppm metal content, 19.00±0.70% total carbon and 3.25±0.50 µmoles m^{-2} surface coverage) was obtained from M/S Spin co tech Pvt. Ltd. Chennai, India. The sample injector was equipped with a 20 µ L loop. For instrument control, data acquisition and processing, LC Solution software was used. The other details of HPLC parameters used for estimation of residues of diafenthiuron are given in Table1.

Table 1: HPLC parameters used for estimation of residues of diafenthiuron

Parameters	
Mobile phase	Acetonitrile
Pump flow (ml/min)	0.30
Pressure (psi)	550
Wave length (nm)	254
Detector	PDA
Column	C_{18}

2.8. Recovery and Limit of Detection

In this study recovery experiments werecarried out at different levels to establish the reliability and validity of analytical method and to know efficacy of extraction and clean-up procedures. The control samples of brinjal and cabbage were spiked at 0.05, 0.10, 0.25, 0.50 and 1.00 mg kg^{-1}, respectively, and processed by following the methodology mentioned in materials and method section. The HPLC chromatogram for standard, control and spiked samples of brinjal and cabbage are represented in Figure 1. and Figure 2, respectively. The average recovery values from the fortified samples were found to be more than 80% (Table 2). The limit of quantification (LOQ) was found to be 0.05 mg kg^{-1} and limit of detection (LOD) being 0.016 mg kg^{-1}.

Table 2: Recovery of diafenthiuron in brinjal fruits and cabbage heads

Substrates	Level of fortification(mg kg^{-1})	Mean recovery (%)
Brinjal	1.00	94.91 ± 3.08
	0.50	90.35 ± 7.78
	0.25	86.33 ± 6.20
	0.10	85.56 ± 5.15
	0.05	84.89 ± 3.17
Cabbage	1.00	96.49 ± 6.50
	0.50	88.23 ± 4.42
	0.25	85.53 ± 3.21
	0.10	85.94 ± 4.53
	0.05	82.99 ± 1.34

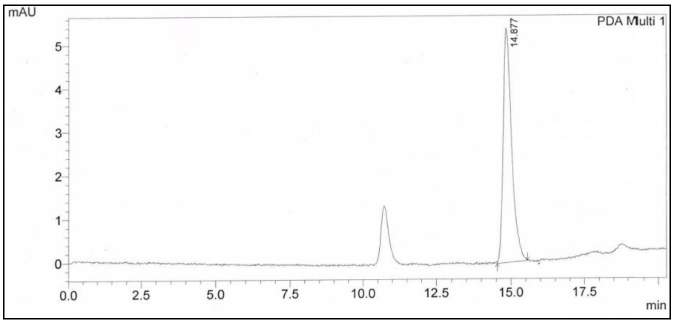


Fig 1: (a) Standard of diafenthiuron

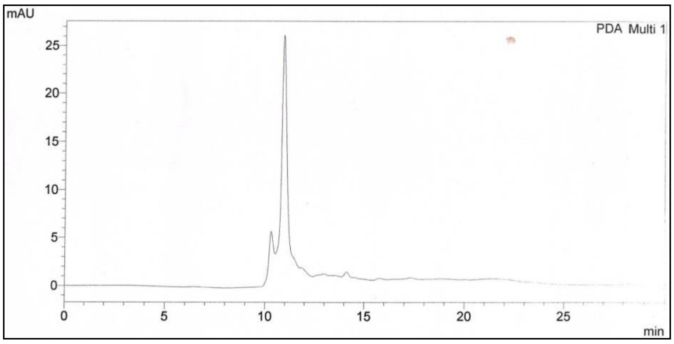


Fig 1: (b) Brinjal control

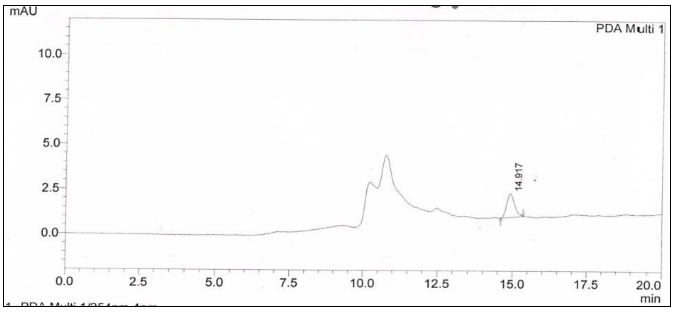


Fig 1: (c) Recovery of spiked brinjal sample @ 0.05 level

Fig 1: HPLC chromatograms of (a) Diafenthiuron Standard (b) brinjal control (c) brinjal spiked sample

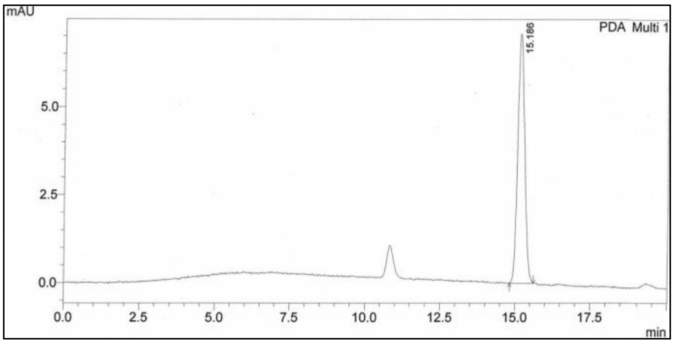


Fig 2: (a) Diafenthiuron Standard for cabbage

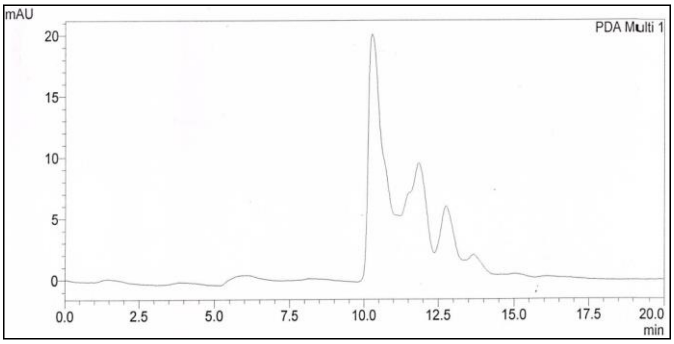


Fig 2: (b) Cabbage-control

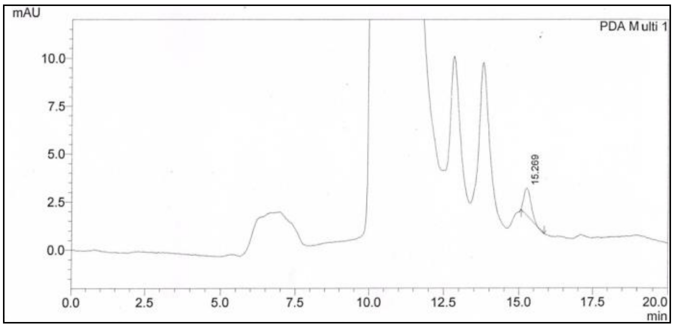


Fig 2: (c) Recovery of spiked cabbage sample @ 0.05 level

Fig 2: HPLC chromatograms of (a) Diafenthiuron Standard (b) cabbage control (c) cabbage spiked sample

3. Results and Discussion

Although all the samples were found contaminated with diafenthiuron residues 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) at recommended dosage. The study revealed that the initial deposits of diafenthiuron on brinjal does not exceed 1 mg kg^{-1} at both single and double dose. However, in case of cabbage samples diafenthiuron residues exceeds MRL at double the recommended dose on 0th day (1 hour after third spray)

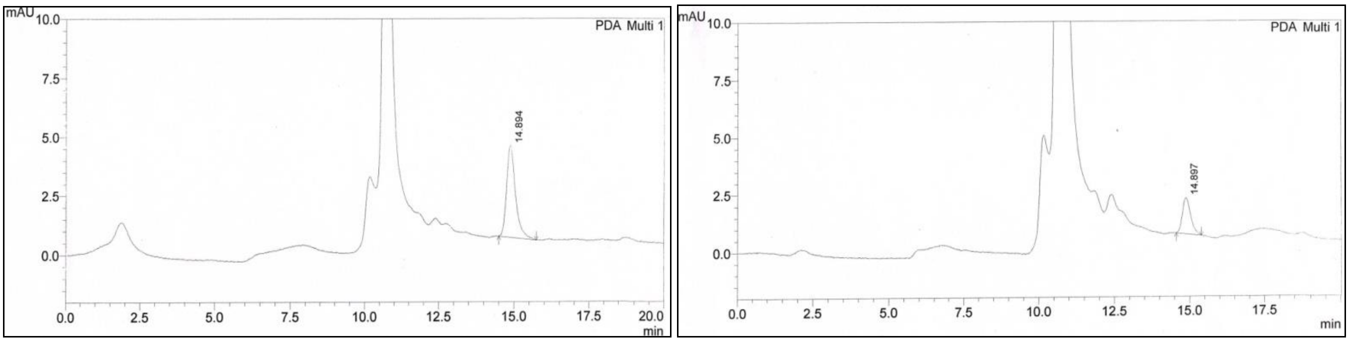
3.1. Brinjal

3.1.1. Washing: The effect of washing of brinjal samples on the reduction of diafenthiuron residues applied @ 300 and 600 g i.e. ha^{-1} from samples of 0 day after the third spray are given in the Table 3. The average initial deposits of 0.26 and 0.60 mg kg^{-1} of diafenthiuron on brinjal fruit samples (Figure

3) Were decreased to 0.05 and 0.09 mg kg^{-1} as a result of normal tap water washing; thus causing a loss of 80.76% and 74.80%, respectively. Mean residues of 0.14 and 0.36 mg $kg^{-1}$of diafenthiuron on brinjal fruits were reached to BDL in case of first day samples of brinjal thereby, leading for a loss of 100% (Table 4, Figure 4) However, Brar *et al* (2017) [14] reported that washing of brinjal treated with tap water reduced the profenofos residues of [1, 3 and 5] day brinjal fruits to 22.10, 30.40 and 43.85 per cent 68.15 to 90.17 per cent. Chandra *et al* (2015) [15] revealed that washing with water removed residues of chlorpyrifos, cypermethrin and monocrotophos by 29.5- 32.5, 29.5-32.5 and 65.6-66.4 per cent respectively. Similarly [16]Kumari in 2008 evaluated the efficacy of various household processes on reducing the residues. It was found that washing effectively removed organ chlorines, synthetic pyrethroids and organ phosphorous insecticide residues of brinjal by [27-44, 26 and 77] per cent respectively.

Table 3: Effect of various processing methods on the reduction of diafenthiuron residues from brinjal (Samples taken 2 h after application)

Processing	Single Dosage			Double Dosage		
	Mean Residue Level ($mgkg^{-1}$)			Mean Residue Level ($mgkg^{-1}$)		
	Before processing	After processing	Reduction %	Before processing	After processing	Reduction %
Washing using Tap water	0.26	0.05	80.76	0.60	0.09	85.00
Boiling	0.26	BDL	100	0.60	BDL	100

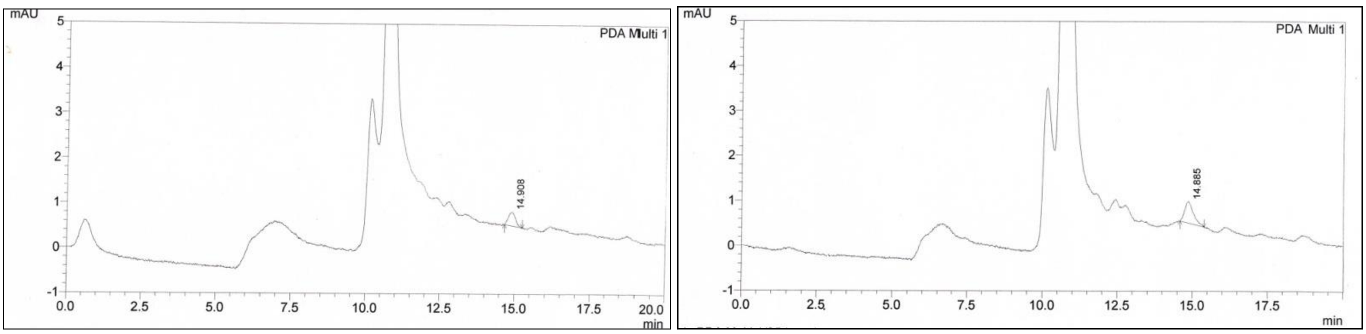


(a) (b)

Fig 3: HPLC chromatogram of 0 the brinjal samples (a) single dose (b) double dose

Table 4: Effect of processing on the reduction of diafenthiuron residues in brinjal (Samples taken 1 day after application)

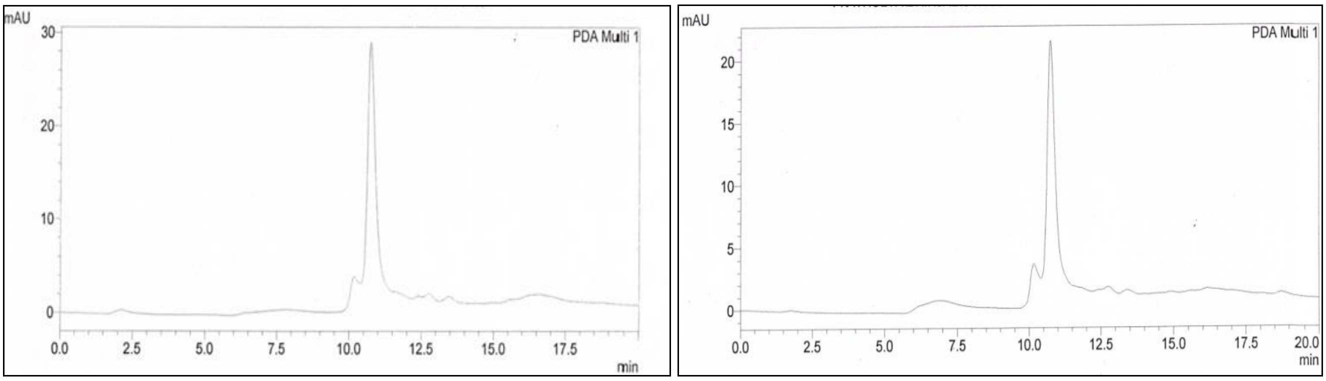
Processing	Single Dosage			Double Dosage		
	Mean Residue Level ($mgkg^{-1}$)			Mean Residue Level ($mgkg^{-1}$)		
	Before processing	After processing	Reduction %	Before processing	After processing	Reduction %
Washing using Tap water	0.14	BDL	100	0.36	BDL	100
Boiling	0.14	BDL	100	0.36	BDL	100



(a) (b)

Fig 4: HPLC chromatogram of 0 the washed brinjal samples (a) single dose (b) double dose

3.1.2 Effect of boiling: The boiling of brinjal showed higher reduction in diafenthiuron residues. The mean initial deposits of 0.26 and 0.60 mg kg^{-1} of diafenthiuron on brinjal fruits samples 2 h after third spray @ 300 and 600 g a.i. ha^{-1} were reduced to BDL thereby, accounting complete removal of residues (Table 3, Figure 5). Similarly in case of 1 day samples, the mean residues of 0.14 and 0.36 mg kg^{-1} of diafenthiuron were reduced also to BDL thereby, accounting 100% removal of residues (Table 4). Walia *et al* (2010) [17] studied the effect of cooking on cypermethrin residue removal in brinjal and found that cooking removed cypermethrin residues by 41.40 per cent. Chauhan *et al* (2011) [18] reported that cooking of brinjal fruits eliminated initial endosulfan residues to 62 and 61.2 per cent in single and double the recommended dose, respectively. Open pan cooking can reduce residues of triazophos, profenofos and ace hate by 50.21-67.27%, 52.63-68.42% and 52.43-56.97%, respectively as reported by Brar *et al* in 2017 [14].`



(a) (b)

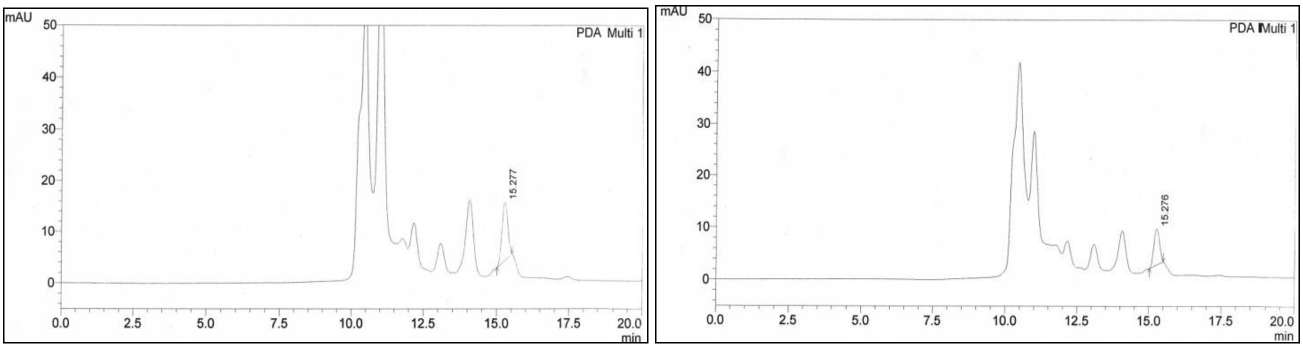
Fig 5: HPLC chromatogram of 0 the boiled brinjal samples (a) single dose (b) double dose

3.2. Cabbage

3.2.1 Washing: The results related to the effect of washing with tap water on the elimination of diafenthiuron applied @300 and 600 g i.e. ha^{-1} from cabbage samples of 0 day after the third spray are represented in Table 5. The average initial deposits of 0.60 and 1.27 mg $kg^{-1}$of diafenthiuronon cabbage samples (Figure 6) were decreased to 0.20 and 0.32 mg kg^{-1} as a result of tap water washing; thus leading to a loss of 66.66% and 74.80%, respectively (Figure 7).But in case of 1 day samples, the average residues of 0.30 and 0.54 mg kg-1 of diafenthiuron on cabbage samples were reduced to BDL and 0.06 mg kg-1 thereby, causing for a loss of 100% and 88.88%, respectively(Table 6).The results of present study is in accordance with that of Gangwar and Singh (1988) [19] reported that washing of cabbage heads treated with carbaryl for 1 to 3 minutes reduced the residues from 68.15 to 90.17 per cent. However, Kar *et al* (2012) [20] reported that the mean initial deposits of cabbage samples collected from the field sprayed with chlorantraniliprole at 9.25 and 18.50 g i.e ha^{-1} were decreased to 0.10 and 0.12 mg kg^{-1} as a result of normal tap water washing; thus causing for a loss of 16.70 and 40.00 per cent, respectively.

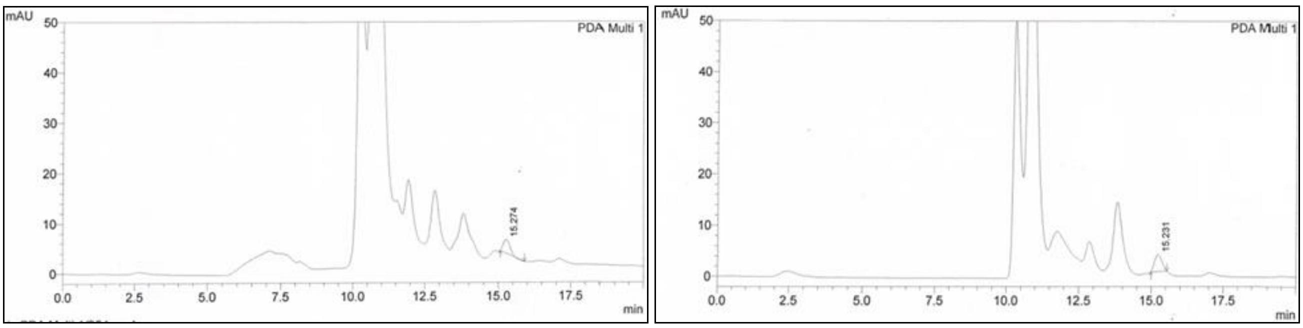
Table 5: Effect of processing on the reduction of diafenthiuron residues in cabbage (Samples taken 2 h after application)

Processing	Single Dosage			Double Dosage		
	Mean Residue Level ($mgkg^{-1}$)			Mean Residue Level ($mgkg^{-1}$)		
	Before processing	After processing	Reduction %	Before processing	After processing	Reduction %
Washing using Tap water	0.60	0.20	66.66	1.27	0.32	74.8
Boiling	0.60	BDL	100	1.27	BDL	100
Removal of outer wrapper leaves	0.60	BDL	100	1.27	BDL	100



(a) (b)

Fig 6: HPLC chromatogram of 0 the cabbage samples (a) single dose (b) double dose



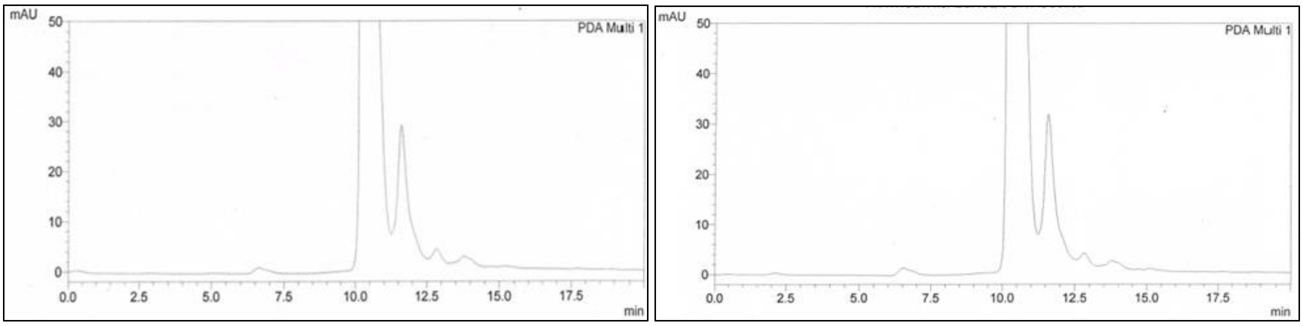
(a) (b)

Fig 7: HPLC chromatogram of washed cabbage samples of 0^{th} day (a) single dose (b) Double dose

3.2.2. Boiling: The boiling of chopped cabbage in water for 2 minutes showed higher drop in diafenthiuron residues. The average initial deposits of 0.60 and 1.27 mg $kg^{-1}$of diafenthiuron on cabbage heads collected 1 hour after the third application of spray @ 300 and 600 g a.i. ha^{-1} were decreased to BDL thereby, resulting complete elimination of residues (Table 5, Figure 8). The average residues 1 daysamples of 0.30 and 0.54 mg $kg^{-1}$of diafenthiuron were also reached to BDL thereby, eliminating 100% of residue removal (Table 6). Nagesh and Verma (1997) [21] reported that washing and cooking effectively reduced the residues of chlorpyriphos and quinalphos in cabbage. Cooking of 0-4 day samples reduced 50 to 70 per cent residues of chlorpyriphos and quinalphos. Kar *et al* (2012) [20] also reported that due to boiling of cabbage heads, mean initial residues of chlorantraniliprole after 0 and 1 day cabbage samples were reduced to BDL. Pareek and Got am (1994) [22] revealed that boiling or cooking of un washed heads of cabbage for15 min reduced methyl-o-demeton residues to the level of 87.39, 80.05, 57.70, 45.45, 83.44 and 100%at 0, 1, 3, 5, 7 and 10 days after insecticide application, respectively.

Table 6: Effect of various processing methods on the reduction of diafenthiuron residues in cabbage (Samples taken 1 day after application)

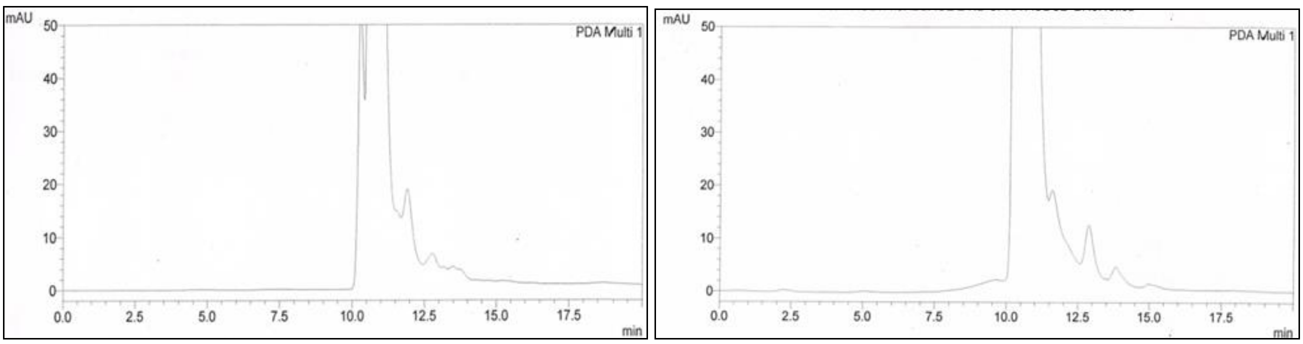
Process	Single Dosage			Double Dosage		
	Mean Residue Level ($mgkg^{-1}$)			Mean Residue Level ($mgkg^{-1}$)		
	Before processing	After processing	Reduction %	Before processing	After processing	Reduction %
Washing using Tap water	0.30	BDL	100	0.54	0.06	88.8
Boiling	0.30	BDL	100	0.54	BDL	100
Removal of outer wrapper leaves	0.30	BDL	100	0.54	BDL	100



(a) (b)

Fig 8: HPLC chromatogram of boiled cabbage samples of 0^{th} day (a) single dose (b) double dose

3.2.3. Removal of outer wrapper leaves: Removing of wrapper leavesof cabbage heads resulted complete removal of insecticide residues (Figure 9). The average initial deposits of 0.60 and 1.27 mg kg^{-1} of diafenthiuron from cabbage heads sampled 1 hour after the third spray were reached to BDL thereby, resulting complete loss (Table 5). In case of cabbage samples collected on first day, where the mean residues of 0.01 and 0.13 mg kg^{-1} were found to below BDL thereby, accounting 100% reduction in both cases (Table 6).The result of present study is in agreement with Kar *et al* (2012) [20] also reported that removal of outer wrapper leaves of cabbage heads, mean initial residues of chlorantraniliprole after 0 and 1 day cabbage samples were reduced to BDL.



(a) (b)

Fig 9: HPLC chromatogram of cabbage samples after removing wrapper leaves (a) single dose (b) double dose

4. Conclusion
Household processing plays an important role in reducing pesticide residues from the food stuff. Pesticide residues in food after harvesting are influenced by various factors like storage, handling, processing etc. Processing at consumer level, prior to consumption can effectively reduce pesticide residues to a great extent. It was found that boiling and washing can effectively reduce the diafenthiuron residues from brinjal and cabbage samples. Removal of outer wrapper leaves completely removed diafenthiuron residue from cabbage. The study also revealed that diafenthiuron residues were found more in cabbage samples than brinjal due to the

smooth surface of latter. Hence, these decontamination studies showed that diafenthiuron at the recommended dose does not pose hazardous effects and is quite safe from environmental point of view.

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