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Decontamination of Chlorantraniliprole Residues on Cabbage and Cauliflower through Household Processing Methods

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Abstract A supervised field trial was conducted to study the residues of chlorantraniliprole on cabbage and cauliflower. Three applications of chlorantraniliprole at 10 days interval were made @ 9.25 and 18.50 g a.i. ha^{-1}. The samples of marketable size heads and curds of cabbage and cauliflower were collected at 0 and 1 day after the last application. QuEChERS sample preparation was used for the determination of chlorantraniliprole residues on cabbage heads and cauliflower curds. The residues of chlorantraniliprole were quantified by high performance liquid chromatography (HPLC) with photo diode array (PDA) detector and confirmed by high performance thin layer chromatography (HPTLC). Washing of cabbage and cauliflower with tap water removed about 17%–40% of chlorantraniliprole residues. However, boiling removed 100% of chlorantraniliprole residues on cabbage and cauliflower in both the cases.

Keywords Boiling · Cabbage · Cauliflower · Chlorantraniliprole · Decontamination · Residues · Washing

Vegetables are the fresh and edible portion of the herbaceous plants. Besides providing bulk and variety to the diet, they are excellent sources of vitamins and minerals essential for human health (Gupta et al. 1998). Cabbage (*Brassica oleracea* var. *capitata* L.) and cauliflower (*Brassica oleracea* var. *botrytis* L.) are two important cole crops of India which are mainly consumed as vegetables in winter season. In India, the area under cabbage and cauliflower were 265.4 and 320.6 thousand hectares with a production of 5,887.8 and 5,796.6 thousand tonnes respectively (Anonymous 2009). In Punjab, the area under cabbage and cauliflower were 3.34 and 6.02 thousand hectares with a production of 73.23 and 138.26 thousand tonnes respectively (Anonymous 2008). The low production of cabbage and cauliflower in the country could be attributed to several factors, the most important being the damage caused by various insect pests. The major insect pests responsible for losses in these crops are stem borer, *Hellula undalis* (Fabricius), diamond-back moth, *Plutella xylostella* (Linnaeus), tobacco caterpillar, *Spodoptera litura* (Fabricius) and aphid, *Lipaphis erysimi* (Kaltenbach). Pesticides (insecticides, fungicides etc.) are used globally for the protection of food and fiber, human health and comfort (Winteringham 1971). However, their excessive use/misuse results in widespread environmental contamination and creating serious acute health problems and local and global environmental impacts (Ecobichon 2001). It is well recognized, that there risks associated with the consumption of pesticide treated crops because of the presence of the toxic residues on them. Therefore, the rational recommendation of a pesticide requires that it must not only provide an effective control of pests, but at the same time its residues on the commodities must be toxicologically acceptable.

Food is the basic necessity of life and food contaminated with toxic pesticides is associated with severe ill effects on the human health. Food safety is of vital importance to the global food supply chain. The recent and growing concern about food safety from public health authorities, the food industry and consumers worldwide has been substantiated by a significant increase in the incidence of reported

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food-borne diseases in many countries during recent years (World Health Organization 2007). Hence, the concern of food safety for developing countries where pesticide consumption is wide spread and a major part of the population lives below the poverty line needs to be taken care. It is therefore of significance to evaluate simple, cost effective strategies to enhance food safety from harmful pesticides for poor populace. Commercial and household processing such as washing, peeling, cooking, blanching, concentrating can reduce residue level in food and then reduce impact on human health (Abou-arab 1999; Byrne and Pinkerton 2004; Soliman 2001; Zohair 2001). Since, no literature pertaining to studies on the effect of processing on the reduction of chlorantraniliprole residues are available, the present studies were planned to study the effect of processing like washing, boiling and removal of outer wrapper leaves on the reduction of chlorantraniliprole residues on cabbage and cauliflower.

Materials and Methods

The technical grade analytical standards of chlorantraniliprole (purity 98.1%) supplied by M/s Sigma Aldrich, India. This formulation (Coragen 18.5 SC) was obtained from M/s E. I. DuPont, India Pvt. Ltd., Gujarat, India. Analysis of acetonitrile extract of the formulation showed only chlorantraniliprole and none of its metabolic product and was found to be accurate with respect to its active ingredient.

Solvents like ethyl acetate, acetonitrile (HPLC grade) and water (HPLC grade) were obtained from Merck, Darmstadt, Germany. Sodium chloride (ASC reagent grade ≥99.9%; NaCl) was also obtained from Merck, Darmstadt, Germany. Sodium sulfate (Na_2SO_4) anhydrous (AR grade) was from S. D. Fine Chemicals, Mumbai. Analytical grade activated anhydrous $MgSO_4$ was also obtained from Merck, Darmstadt, Germany. Primary Secondary Amine (PSA) Sorbent and activated graphitized carbon black (GCB, 400 mesh) were obtained from Sigma-Aldrich, Mumbai, India. All common solvents were redistilled in all-glass apparatus before use. The suitability of the solvents and other chemicals were ensured by running reagent blanks before actual analysis.

A standard stock solution of chlorantraniliprole (1 mg/mL) was prepared in HPLC grade acetonitrile. The standard solutions required for constructing a calibration curve (2.00, 1.50, 1.00, 0.50, 0.25 and 0.10 μg/mL) were prepared from stock solution by serial dilution with HPLC grade acetonitrile. All standard solutions were stored at 4°C before use.

Cabbage (var. Pride of India) and cauliflower (var. Giant Snowball) were raised at Entomological Research Farm, Punjab Agricultural University, Ludhiana following recommended agronomic practices (Anonymous 2008). There were three treatments i.e. control, recommended and double the recommended dosages and three replications for each treatment arranged in a randomized block design (RBD), and size of the each plot was 50 m^2.

The first application of chlorantraniliprole (Coragen 18.5 SC) @ 9.25 and 18.50 g a.i. ha^{-1} was made at head formation stage followed by another two applications at 10 days interval. Pesticide was sprayed as foliar application with the help of a ASPEE Knapsack sprayer fitted with hollow cone nozzle.

About 1 kg heads/curds of marketable size were collected randomly 0 (1 h) and 1 days after the third application of the insecticide. The cabbage heads and cauliflower curds were collected from each plot separately, packed in polyethylene bags and brought to the laboratory for processing. Samples were processed immediately after sampling.

In treatment one (T_1), each replicated cabbage heads and cauliflower curd samples (100 g) was washed under running tap water for 2–3 min. In the second treatment (T_2), the heads were cooked in boiling water (500 mL for each 100 g sample) for 5 min, and the water was discarded. The next treatment (T_3) was the combination of the above two, i.e., heads (100 g) were washed thoroughly under tap water for 2–3 min followed by boiling in 500 mL water for 5 min, and the water was discarded. In the next treatment (T_4), the outer wrapper leaves of cabbage were removed and then cut into small pieces.

The cabbage and cauliflower samples were prepared following QuEChERS method for the determination of chlorantraniliprole residues. A sub sample of 15 g of cabbage heads cauliflower curds was weighed into a 50 mL centrifuge tube and then 30 mL distilled ethyl acetate was added to it. The sample was homogenized using high speed homogenizer (Heidolph Silent Crusher-M®) for 2–3 min at 1,400–1,500 rpm. Sodium chloride (NaCl) 10 ± 0.1 g was added to homogenized sample for phase separation. The contents were centrifuged at 2,500–3,000 rpm for 3 min. An aliquot of 15 mL ethyl acetate layer was transferred over 10 ± 0.1 g pre-activated sodium sulfate (Na_2SO_4) in a test tube. The ethyl acetate extract subjected to cleanup by dispersive solid phase extraction (DSPE). An aliquot of 6 mL ethyl acetate was taken in a test tube containing 0.15 ± 0.01 g PSA sorbent, 0.90 ± 0.01 g anhydrous $MgSO_4$ and 0.05 ± 0.01 g graphitic carbon black and the content was thoroughly mixed on vortex shaker. Again it was centrifuged at 2,500–3,000 rpm for 1 min. 4 mL aliquot of this ethyl acetate extract was evaporated to dryness using low volume evaporator to dryness using low volume evaporator at 35°C. Volume was made up to 2 mL with HPLC grade distilled acetonitrile.

The quantification of chlorantraniliprole residues were done by using high performance liquid chromatograph

(HPLC). The high performance liquid chromatograph (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 analyses were carried out at column temperature 25°C under isocratic condition acetonitrile:water (80:20, v/v) with pump flow @ 0.400 mL min^{-1}. The instrument was set at wavelength of 254 nm. An injection volume of 20 µL was used in all experiments. Residues of chlorantraniliprole were quantified by comparison of peak height/peak area of standards with that of unknown or spiked samples run under identical conditions. Under these operating conditions the retention time of chlorantraniliprole was found to be 9.86 min.

The confirmation of chlorantraniliprole residues was done by using high performance thin layer chromatography (HPTLC). High performance thin layer chromatograph was from M/S CAMAG, Switzerland. Parts of the instruments are Linomat-5 applicator, Camag-Hamilton Linomat syringae (100 µL), Camag ADC 2 Automatic Developing Chamber, UV lamp with cabinet; TLC scanner 3 with winCATS version 1.4.2.8121 evaluation software (Camag, Muttenz, Switzerland). Precoated silica gel (60 F_{254}) TLC aluminum sheets were obtained from Merck, Darmstadt, Germany. Bands of 8 mm length and 6 tracks were applied on each pre-coated TLC plate (10 cm × 10 cm). Linomat-5 applicator was programmed so that bands were applied at a distance of 10 mm from the bottom and at least 12 mm distance between tracks. Distance between tip of the syringe and TLC plate was fixed at 1 mm for sharp application of bands. Samples were applied on the TLC plate at the delivery rate of 200 nL/s and nitrogen gas used for drying the spots. Loaded plate was viewed inside the cabinet under UV light to ensure proper application before development. Loaded TLC plates were developed in Camag-Automatic development chamber up to 8 cm in a paper lined chamber pre saturated with 25 mL of ethyl acetate. The chlorantraniliprole bands on developed plate were quantitated by absorbance at single wavelength in the Camag TLC scanner 3 using *winCATS* software. Scanning parameters were deuterium lamp wavelength 270 nm, Slit length 6.00 mm, slit width 0.3 mm, band width 30 nm, micromode. Under these conditions, chlorantraniliprole showed a relative front (R_f) value of 0.54. Each band was quantified in a single beam, single wavelength reflectance mode, and relative front was taken into consideration for confirmation of residues of chlorantraniliprole.

Results and Discussion

In the present investigations recovery experiments were carried out at different levels to establish the reliability and validity of analytical method and to know the efficiency of extraction and clean-up procedures. The control samples of cabbage and cauliflower were spiked at 0.10, 0.25, 0.50 and 1.00 mg kg^{-1}, respectively, and processed by following the methodology as described above. The average recovery values from the fortified samples were found to be more than 85% (Table 1). The limit of quantification (LOQ) was found to be 0.10 mg kg^{-1} and limit of detection (LOD) being 0.03 mg kg^{-1}.

Cabbage

Effect of washing: The results pertaining to the effect of washing on the removal of chlorantraniliprole applied @ 9.25 and 18.50 g a.i. ha^{-1} from 0 day samples of cabbage after the third spray are presented in Table 2. The mean initial deposits of 0.12 and 0.20 mg kg^{-1} of chlorantraniliprole on cabbage samples were reduced to 0.10 and 0.12 mg kg^{-1} as a result of simple washing with tap water; thus accounting for loss of 16.70% and 40.00%, respectively. But in case of 1 day samples, the mean residues of 0.10 and 0.13 mg kg^{-1} of chlorantraniliprole on cauliflower samples were reduced to BDL and 0.10 mg kg^{-1} thereby, accounted for loss of 100% and 23.08%, respectively (Table 3). These results are in agreement with those of Nagesh and Verma (1997) who studied the decontamination of cabbage treated with chlorpyriphos and quinalphos. Washing of the vegetable treated with chlorpyriphos @ 0.05% reduced initial deposits from 13.81 to 8.56 mg kg^{-1}, showing a reduction of 38.02%. For quinalphos, though reduction of residues was 39.06%–44.0%, but it did not help much in bringing the residues below MRL of 0.25 mg kg^{-1}.

Effect of boiling: The boiling of cabbage showed higher reduction in chlorantraniliprole residues. The mean initial deposits of 0.12 and 0.20 mg kg^{-1} of chlorantraniliprole on cabbage samples collected after 1 h after the third spray

Table 1 Recovery of chlorantraniliprole in cabbage heads and cauliflower curds

Substrates	Level of fortification (mg kg^{-1})	Mean recovery (%)
Cabbage heads	0.10	91.65 ± 2.87
	0.25	87.46 ± 1.43
	0.50	94.50 ± 1.78
	1.00	90.18 ± 3.56
Cauliflower heads	0.10	90.25 ± 1.89
	0.25	89.76 ± 2.43
	0.50	87.89 ± 2.07
	1.00	93.50 ± 3.45

[a] Each value is mean ± SD of three replicate determinations

Table 2 Effect of processing on the reduction of chlorantraniliprole residues in cabbage (Samples taken 1 h after application)

Process	Single dose			Double dose		
	Mean residue level (mg kg^{-1})[a]		Reduction (%)	Mean residue level (mg kg^{-1})		Reduction (%)
	Before	After		Before	After	
Washing	0.12 ± 0.01	0.10 ± 0.01	16.70	0.20 ± 0.02	0.12 ± 0.01	40.00
Boiling	0.12 ± 0.01	BDL	100.00	0.20 ± 0.02	BDL	100.00
Washing and boiling	0.12 ± 0.01	BDL	100.00	0.20 ± 0.02	BDL	100.00
Removal of outer wrapper leaves	0.12 ± 0.01	BDL	100.00	0.20 ± 0.02	BDL	100.00

BDL below detectable limit (< 0.10 mg kg^{-1})

[a] Each value is mean ± SD of three replicate determinations

Table 3 Effect of processing on the reduction of chlorantraniliprole residues in cabbage (Samples taken 1 day after application)

Process	Single dose			Double dose		
	Mean residue level (mg kg^{-1})[a]		Reduction (%)	Mean residue level (mg kg^{-1})		Reduction (%)
	Before	After		Before	After	
Washing	0.10 ± 0.01	BDL	100.00	0.13 ± 0.02	0.10 ± 0.01	23.08
Boiling	0.10 ± 0.01	BDL	100.00	0.13 ± 0.02	BDL	100.00
Washing and boiling	0.10 ± 0.01	BDL	100.00	0.13 ± 0.02	BDL	100.00
Removal of outer wrapper leaves	0.10 ± 0.01	BDL	100.00	0.13 ± 0.02	BDL	100.00

BDL below detectable limit (< 0.10 mg kg^{-1})

[a] Each value is mean ± SD of three replicate determinations

@ 9.25 and 18.50 g a.i. ha^{-1} were reduced to BDL thereby, accounting complete removal of residues (Table 2). Similarly in case of 1 day samples, the mean residues of 0.10 and 0.13 mg kg^{-1} of chlorantraniliprole were reduced to BDL also (Table 3). Pareek and Gotam (1994) reported that the unwashed cabbage heads when boiled or cooked for 15 min decontaminated the residues of methyl-o-demeton to the extent of 87.39, 80.05, 57.70, 45.45, 83.44 and 100% at 0, 1, 3, 5, 7 and 10 days after application of insecticides, respectively.

Effect of washing and boiling: Washing followed by boiling also resulted complete removal of chlorantraniliprole residues as compared to washing alone. The mean initial deposits of 0.12 and 0.20 mg kg^{-1} of chlorantraniliprole on cabbage samples collected 1 h after the third spray @ 9.25 and 18.50 g a.i. ha^{-1} were reduced to BDL thereby, accounting complete loss (Table 2). Same scenario was also found in case of 1 day samples where the mean residues of 0.10 and 0.13 mg kg^{-1} of chlorantraniliprole were found to reduce to BDL thereby, showing 100% reduction in both cases (Table 3). These results are in agreement with those of Aktar et al. (2010) who reported that washing under running tap water removed on an average 27.72%–32.48% of quinalphos residues from cabbage head. After cooking, this reduction was 41.30%–45.20%, and in washing plus cooking, it further increased to a range of 66.45%–68.19%.

Effect of removal of outer wrapper leaves: Removal of outer wrapper leaves also resulted complete reduction of pesticide residues. The mean initial deposits of 0.12 and 0.20 mg kg^{-1} of chlorantraniliprole on cabbage samples collected 1 h after the third spray were reduced to BDL thereby, accounting complete loss (Table 2). In case of 1 day sample where the mean residues of 0.01 and 0.13 mg kg^{-1} were found to be reduced to BDL thereby, showing 100% reduction in both cases (Table 3).

Cauliflower

Effect of washing: The results pertaining to the effect of washing on the removal of chlorantraniliprole residues applied @ 9.25 and 18.50 g a.i. ha^{-1} from 0 day samples of cauliflower after the third spray are presented Table 4. The mean initial deposits of 0.18 and 0.29 mg kg^{-1} of chlorantraniliprole on cauliflower samples were reduced to 0.13 and 0.18 mg kg^{-1} as a result of simple washing with tap water; thus accounting for loss of 27.80% and 38.00%, respectively. But in case of 1 day samples, the mean residues of 0.11 and 0.15 mg $kg^{-1}$of chlorantraniliprole on

Table 4 Effect of processing on the reduction of chlorantraniliprole residues in cauliflower (samples taken 1 h after application)

Process	Single dose			Double dose		
	Mean residue level (mg kg^{-1})[a]		Reduction (%)	Mean residue level (mg kg^{-1})		Reduction (%)
	Before	After		Before	After	
Washing	0.18 ± 0.01	0.13 ± 0.01	27.80	0.29 ± 0.03	0.18 ± 0.02	38.00
Boiling	0.18 ± 0.01	BDL	100.00	0.29 ± 0.03	BDL	100.00
Washing and boiling	0.18 ± 0.01	BDL	100.00	0.29 ± 0.03	BDL	100.00

BDL below detectable limit (< 0.10 mg kg^{-1})

[a] Each value is mean ± SD of three replicate determinations

cauliflower were reduced to BDL and 0.11 mg kg^{-1} thereby, accounted for loss of 100% and 26.70%, respectively (Table 5). However, Dhiman et al. (2006) reported that washing of cauliflower treated with chlorpyriphos, quinalphos, endosulfan, fenvalerate and deltamethrin reduced 28.92%–78.64% residues of these insecticides. In contrast, a reduction of 70% in endosulfan residues was reported when cauliflower curds were washed with running tap water for 2 min.

Effect of boiling: The boiling of cauliflower showed higher reduction in chlorantraniliprole residues. The mean initial deposits of 0.18 and 0.29 mg kg^{-1} of chlorantraniliprole on cauliflower samples 1 h after third spray @ 9.25 and 18.50 g a.i. ha^{-1} were reduced to BDL thereby, accounting complete removal of residues (Table 4). Similarly in case of 1 day samples, the mean residues of 0.11 and 0.15 mg kg^{-1} of chlorantraniliprole were reduced also to BDL (Table 5). Fernandez-Cruz et al. (2006) studied captan and fenitrothion dissipation in field-treated cauliflower and effect of household processing. He observed that washing did not significantly affect the residual amounts of captan and fenitrothion in raw vegetable; however, after cooking, captan had degraded completely, whereas residue levels of fenitrothion were not modified significantly. A reduction of 75% endosulfan residues in cauliflower after cooking was observed by Dinabandhoo and Sharma (1994).

Effect of washing and boiling: Washing followed by boiling also resulted complete reduction in chlorantraniliprole residues as compared to washing alone. The mean initial deposits of 0.18 and 0.29 mg kg^{-1} of chlorantraniliprole on cauliflower samples collected 1 h after the third spray @ 9.25 and 18.50 g a.i. ha^{-1} were reduced to BDL thereby, accounting complete loss of residues, respectively (Table 4). Same scenario was also found in case of 1 day samples where the mean residues of 0.11 and 0.15 mg kg^{-1} of chlorantraniliprole were found to reduce to BDL thereby, showing 100% reduction in both cases (Table 5). However, Mukherjee et al. (2006) studied the reduction of chlorpyriphos residues from contaminated cauliflower curds by effect of washing, cooking, washing + cooking, salt water dipping, dipping in boiled salt water, dipping in detergent solution and dipping in boiled detergent solution. The analysis showed that various food processing techniques substantially lowered the residue of chlorpyriphos in cauliflower curds from 27.9% to 73.3% but none was able to satisfactorily bring down the residues below the tolerance level of 0.01 μg g^{-1}. Cooking after washing mitigated 5 days old residues by 94.49%, 37.97% and 11.64% from recommended rates of application of endosulfan, fenvalerate and monocrotophos, respectively (Dinabandhoo and Sharma 1994).

Use of pesticides certainly leaves certain amount of residues which may prove hazards to the health of the

Table 5 Effect of processing on the reduction of chlorantraniliprole residues in cauliflower (samples taken 1 day after application)

Process	Single dose			Double dose		
	*Mean residue level (mg kg^{-1})		Reduction (%)	Mean residue level (mg kg^{-1})		Reduction (%)
	Before	After		Before	After	
Washing	0.11 ± 0.01	BDL	100.00	0.15 ± 0.01	0.11 ± 0.01	26.67
Boiling	0.11 ± 0.01	BDL	100.00	0.15 ± 0.01	BDL	100.00
Washing and boiling	0.11 ± 0.01	BDL	100.00	0.15 ± 0.01	BDL	100.00

BDL below detectable limit (< 0.10 mg kg^{-1})

[a] Each value is mean ± SD of three replicate determinations

consumers. Though remedial measures like safe waiting period reduces the risk of the residue problems, processing like washing and boiling may further reduce the risk and make the commodities toxicologically acceptable to the consumers. Among various decontamination methods for cauliflowers, washing, boiling, washing + boiling are effective in significantly decreasing the intake of pesticide residues from cabbage and cauliflower. It is suggested that cabbage and cauliflower should be thoroughly washed and boiled before consumption.

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