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Full Paper

Degradation dynamics and risk assessment of chlorpyriphos in/ on cabbage under different culinary processes

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ABSTRACT: *Chlorpyriphos 20 EC was applied at the rate of 500 g a.i. ha^{-1} in Cabbage heads and the samples harvested at intervals of 0 (2 hours after application), 1 and 7 days after application. The calculated half-life value and safe waiting period (8.75 and 45.29 days respectively), indicated its longer persistence. Thus, to reduce the safe waiting period, efforts were made to decontaminate the Chlorpyriphos residue from Cabbage head by various household preparations (viz. washing, cooking, washing plus cooking, salt water dipping, dipping in boiled salt water, dipping in detergent solution and dipping in boiled detergent solution). Statistical analysis of the data using Duncan's Multiple Range Test revealed that various household processing substantially reduced the residue of Chlorpyriphos in Cabbage heads in the range of 27.89-73.32 % but none were able to satisfactorily bring down the residue below the tolerance level of 0.05 mg kg^{-1}. A minimum of about twelve days was suggested as safe waiting period.*

Keywords: *chlorpyriphos, residues, decontamination, cabbage, household preparations*

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Introduction

Cabbage (*Brassica oleracea* var. capitata), an important winter vegetable crop grown in India, with an annual production of 3.39 million tons and consumption of ~9.3 g/day, is heavily attacked by many organisms, including diamond back moth (*Plutella xylostella*), leaf eating caterpillar and aphids, resulting in severe loss of quality and production [1, 2]. Chlorpyriphos [*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate] is a contact insecticide intensively used by the farmers in many parts of West Bengal as a plant protection measure. Surveys conducted in India have indicated that 50-70% of vegetables are contaminated with the insecticide residues probably due to their use in the field [3, 4]. Contamination may have occurred mainly due to harvest the crops before the recommended waiting period. In general pesticide analyses are performed in raw agricultural commodity, which including the peel and (other) non-edible parts. However, Cabbages are subjected to some form of household preparations, e. g. washing, cooking, removal of non-edible parts etc. before actual consumption. Some studies have shown that certain types of postharvest treatments or household preparations may help to reduce pesticide residues [5-8]. The effects of these processing techniques on residue levels are extremely important in evaluating the risk associated with ingestion of pesticides residues. To date, very little information is available on the influence of food processing on a specific pesticide-commodity combination and this is important because the behavior and fate of the chemical varies with the pesticide as well as with the crop. The data regarding the effect of various household preparations in reduction of Chlorpyriphos residues in Cabbage is scanty. Therefore, the present investigation was carried out with the objective to examine the persistence of Chlorpyriphos on Cabbage heads and to evaluate the impact of various household preparations (washing, cooking, washing plus cooking, salt water washing, detergent washing) in reduction of Chlorpyriphos residues from Cabbage heads.

Material and Methods

Field Experiment and Collection of Samples

The experiment was conducted in Cabbage (variety Indian Rare ball) at the Agricultural Research Farm, Baruipur under the operational area of Institute of Agricultural Science, University of Calcutta, Kolkata, West Bengal, India, during September-December, 2005. Chlorpyriphos 20 EC was purchased from the local market and applied at the rate of 500 g a.i. ha^{-1} during 50 % fruiting (cabbage head) stage. The formulation was diluted with water and sprayed at 600 L ha^{-1} with a knapsack sprayer.

Samples of Cabbage heads (~20 kg) were drawn randomly from the whole field (90 m^2) at 0 (2 h after application), 1 and 7 days after spraying, packed into brown paper bags and brought to laboratory, cut into small pieces, mixed thoroughly and sub-samples (3 x 100 g) were weighed for fresh heads for each household processing treatments.

Household Preparation

In treatment one, each replicated sample (100 g) was washed under running tap water for 2 minutes (T_1). In the second treatment, the heads were cooked in boiling water (500 mL for each 100 g sample) for 5 minutes and the water was discarded (T_2). The next treatment is the combination of the above two (T_3), i.e. heads (100 g) were washed thoroughly under tap water for 2 minutes followed by boiling in 500 mL water for 5 minutes and the water was discarded. In the fourth and fifth treatments, the heads were dipped in 500 mL 2% salt brine solution at room temperature, (28 ± 1 ^{0}C, T_4) and at hot (85 ± 1 ^{0}C, T_5) condition for 5 minutes and washed under tap water for 2 minutes. In the sixth and seventh treatments, this procedure was applied using 1% detergent solution (pH 10.2) at room temperature (T_6) and at hot (T_7) for 2 minutes followed by 5 minutes wash. The field samples analyzed without any household technique are designated as unprocessed control (T_0).

Extraction and clean-up of residues

The unwashed and the various home processed cabbage samples were blended separately in a Remi–Automix blender for two minutes with 150 mL acetone, filtered through Whatman no. 1 filter paper, concentrated (~20 mL) using rotary vacuum evaporator, partitioned thrice with dichloromethane (100 + 50 + 50 mL), combined and concentrated to ~10 mL and the organic phase was further cleaned up using C-18 silica gel Solid Phase Extraction (SPE) column (1 g, 6 cc, Varian). The SPE column was eluted with 200 mL ethyl acetate, evaporated to dryness, and the volume made up with distilled hexane (10 mL) for gas chromatographic analysis.

Estimation of residues

An aliquot (1 μL) of cleaned up extract was injected into gas chromatograph with 10 μl Hamilton Syringe™. The residues of Chlorpyriphos in samples were identified by comparing the retention time of the sample peaks with the standard (99.5% purity, Sigma-Aldrich) solution containing 1 ppm of Chlorpyriphos. The residues of Chlorpyriphos were analyzed on GC (Agilent Technologies 6890N Network GC system) with electron capture detector (ECD-Source Ni^{63}) coupled with Chemito 5000 data processor. The HP-5 capillary column (30 m x 0.32 mm i.d.) of 0.25 μm film thickness was used. The temperatures were: Oven 210 ^{0}C, Injector 230 ^{0}C, Detector 300 ^{0}C. Flow rate of carrier gas (Nitrogen, purity 99.97%) was 2 mL min^{-1} and make up gas (Nitrogen, purity

99.97%) was 60 mL min^{-1}. The standardization was done using calibration curves (external standard). The retention time, limit of detection (LOD) and limit of quantification (LOQ) were 5.38 min, 0.01 µg g^{-1} and 0.05 µg g^{-1}, respectively.

Calculation of Residues

The residue content was calculated by using the formula

$$\text{Residue in ppm (µg/g)} = \frac{A_1 \times C \times V_1}{A_2 \times W \times V_2} \times R_f$$

Where,

A_1 = Area of Oryzalin from sample, in chromatogram

A_2 = Area of Oryzalin from standard, in chromatogram

V_1 = Total volume of sample (in mL)

C = Concentration of analytical standard in ppm (µg/mL) x µL injected

W = Weight of the sample (in gm)

V_2 = Injected volume of sample (in µL)

R_f = Recovery factor

Linearity was evaluated by linear regression analysis

Recovery Studies

In order to estimate the efficiency of the method, a recovery experiment was conducted by fortifying untreated samples with analytical grade Chlorpyriphos (99.5% purity, Sigma-Aldrich™) at the rate of 0.25, 0.50 and 1.00 µg g^{-1} level. The fortified samples were analyzed and estimated following the method described earlier. Recovery of the method was obtained in the range of 93-98% with an average of 95.67% (Table 1). The limit of quantification (LOQ) of the method was 0.05 mg Kg^{-1}.

Table 1. Results of method validation by recovery analysis of Chlorpyriphos (analytical grade) from Cabbage heads.

Substrates	Amount fortified (µg g^{-1})§	Amount recovered (µg g^{-1}) §	Recovery (%)	Average recovery (%)
Cabbage heads	0.25	0.23	93	95.67
	0.50	0.48	96	
	1.00	0.98	98	

§ Average of three replicates

Statistical analysis

The residue data were subjected to statistical analysis by using regression equations and half-life ($T_{1/2}$) values. The ($T_{1/2}$) values obtained were further tested by one-way analysis of variance (ANOVA) using SPSS 10.0 statistical package. The Duncan's Multiple Range Test (DMRT) was used to determine the statistical significance of various home processing treatments in reducing Chlorpyriphos residues using the same package.

Results and Discussion

Persistence of Chlorpyriphos

The initial residues of Chlorpyriphos in Cabbage heads after 2 hours (0 day) of spray was found to be 2.91 mg Kg^{-1} (Table 2). After 1 and 7 days, residues declined to 2.45 and 1.61 mg Kg^{-1} respectively showing a reduction of 15.81 and 44.67%. The dissipation of Chlorpyriphos residues followed first order reaction kinetics. The calculated half–life ($T_{½}$) value was found to be 8.75 days, indicating its persistence nature. The low temperature in winter season might have led to slow dissipation of Chlorpyriphos residues in Cabbage heads.

Effect of various household preparations

Washing Cabbage head under running tap water (T_1) removed an average 27.89% of Chlorpyriphos residues from head (Table 2). After cooking (T_2), this reduction was 41.40% and in washing plus cooking (T_3) it further increased to 66.78%. This seems to suggest that the loosely bind surface residue may be removed by washing with water; while the Chlorpyriphos that may have penetrated into the surface of the heads might not be appreciably reduced by cooking for 5 minutes in boiling water. It was also observed that separate treatments of washing and cooking (i.e. T_1 +T_2) reduced a total of 69.29% Chlorpyriphos residues. It was close to the treatments of washing and cooking in succession (T_3) caused 66.78% reduction of residues. The half-life values of Chlorpyriphos reduced from 8.75 to 3.85 days in case of the home processing treatment (T_3) and this value was significantly lower than T_1 and/or T_2 at 5% level of significance (Table 2). Thus, washing or cooking alone did not enhance much the reduction residues; instead washing followed by cooking seems to have the reduction of residues.

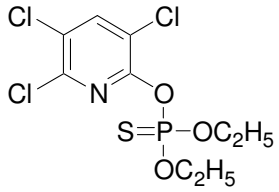
Dipping in 2% brine solution (T_4) followed by washing reduced the residues by 39.58% while in the case of hot 2% brine solution (T_5) this reduction was 55.01%. However, T_4 did not differ significantly of T_2. Similar type of observation with malathion, quinalphos and chlorpyriphos in cabbage were reported in published literatures [9-11].

Table 2. Residues of Chlorpyriphos in Cabbage heads and its removal by Household Preparations.

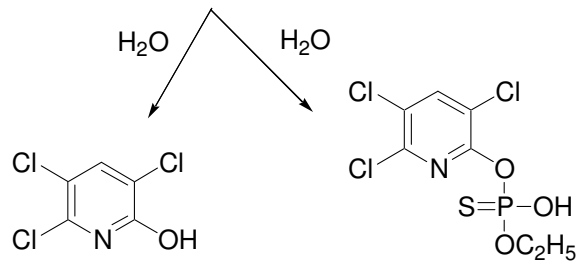
Treatments	Residues in mg/kg * (±SD) at different days interval			Mean % reduction	Mean $T_{½}$ days**
	0	1	7		
T_0	2.91 (± 0.02)	2.45 (± 0.03)	1.61 (± 0.05)	-	8.75$^{\Omega}$
T_1	2.39 (± 0.01)	1.49 (± 0.01)	1.19 (± 0.01)	27.89	8.85 $^{\Omega}$
T_2	1.94 (± 0.03)	1.19 (± 0.16)	0.98 (± 0.04)	41.40	9.29 $^{\Omega}$
T_3	1.59 (± 0.08)	0.60 (± 0.03)	0.33 (±0.01)	66.78	3.85 ▪
T_4	1.79 (± 0.04)	1.47 (± 0.04)	0.96 (± 0.04)	39.58	8.46 $^{\Omega}$
T_5	1.74 (± 0.03)	0.84 (± 0.02)	0.66 (± 0.07)	55.01	6.73 ◉
T_6	0.92 (± 0.05)	0.63 (± 0.06)	0.36 (± 0.01)	73.32	5.85 ◉
T_7	1.34 (± 0.02)	0.58 (± 0.07)	0.27 (± 0.03)	71.18	3.57 ▪

* Average of three replications ** Similar subscripts signify homogeneous means due to DMRT

Dipping cabbage in 1% detergent (T_6 and T_7) followed by thorough washing reduced the residues by 73.32 and 71.18 % respectively, thereby showing the effectiveness of detergent wash in Chlorpyriphos removal. It was 2.6 times more efficient compared to water wash. The hydrolysis of Chlorpyriphos occurs readily at pH > 7 (Fig. 1) [12], which might arise due to detergent solution, resulting in higher removal of Chlorpyriphos. The degradation of Chlorpyriphos with time under different culinary process is presented in Fig. 2.



Chlorpyriphos



3,5,6-trichloropyridinol *O*-ethyl-*O*-(3,5,6-trichloro-2-pyridyl)thiophosphate

Figure 1. Probable degradation of Chlorpyriphos at pH > 7.

Dietary risk assessment of Chlorpyriphos (Table 3) revealed no appreciable risk arising through Cabbage consumption. The dietary intake of Chlorpyriphos was found in the range of 0.16–0.50 μg Kg^{-1} body weight on 0, 1 and 7 days contributing only a

meager 1.58–5.01 % of Acceptable Daily Intake or ADI (0.01 mg Kg^{-1}) on 0 day. The percent contribution was further decreased to 1.01–4.23 and 0.46–2.78 % on 1 and 7 days respectively irrespective of the type of household treatment followed. Although the consumption of Cabbage alone does not represent any risk to consumers but considering the wide range of Chlorpyriphos use on various crops [13-15] may contribute a significant amount of Chlorpyriphos intake through total diet.

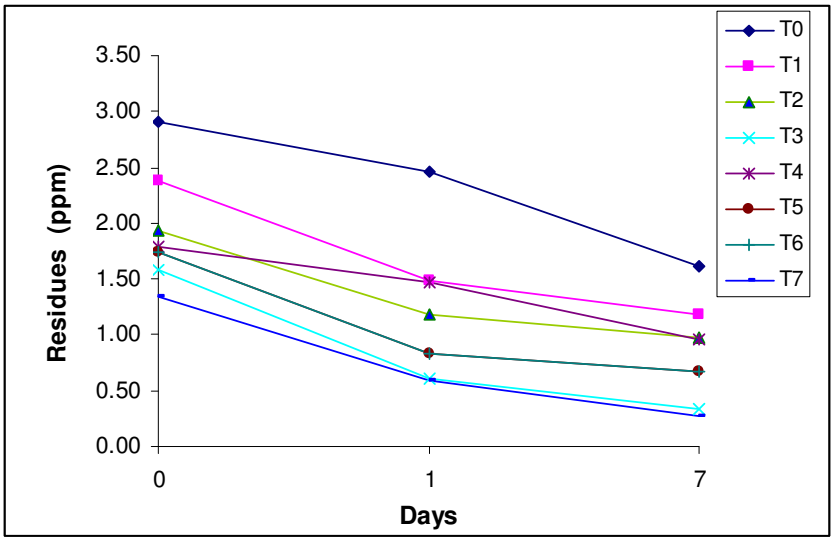


Figure 2. Degradation of Chlorpyriphos under different culinary process

Table 3. Dietary exposure of Chlorpyriphos through consumption of Cabbage heads.

Treatments	Dietary intake (DI, µg/kg body weight) and % ADI of Chlorpyriphos on different days							
	0		1		7		Mean	
	DI	% ADI	DI	% ADI	DI	% ADI	DI	% ADI
T_0	0.50	5.01	0.42	4.23	0.28	2.78	0.45	4.51
T_1	0.41	4.12	0.26	2.56	0.20	2.04	0.33	3.27
T_2	0.33	3.34	0.20	2.04	0.17	1.69	0.27	2.65
T_3	0.27	2.74	0.10	1.04	0.06	0.57	0.16	1.63
T_4	0.31	3.08	0.25	2.53	0.17	1.66	0.27	2.73
T_5	0.30	2.99	0.14	1.44	0.11	1.14	0.21	2.09
T_6	0.16	1.58	0.11	1.09	0.06	0.63	0.12	1.24
T_7	0.23	2.30	0.10	1.01	0.05	0.46	0.14	1.42

Conclusion

Thus, a comparison of the overall effects of different household preparations indicated that levels of Chlorpyriphos residues can be reduced significantly by mild detergent washing or by washing plus cooking. The reduction in residue levels makes

these procedures worthwhile for adoption by the consumer. The effectiveness of different treatments was observed in the order of $T_3 \approx T_7 > T_6 \approx T_5 > T_4 \approx T_1 \approx T_2 \approx T_0$. Strong adsorption properties coupled with poor water solubility of Chlorpyriphos might be responsible for reducing the efficiency of the home processes for decontaminating the Cabbage heads. Hence to reduce the risk associated with intake of Chlorpyriphos through Cabbage heads, washing plus cooking or mild detergent washing procedures should be followed before consumption.

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