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Decontamination Processing of Chlorpyrifos and Cypermethrin Residues in Cauliflower

Tanuja Banshtu* and Surender Kumar Patyal

Department of Entomology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan- 173 230 Himachal Pradesh, India

**Corresponding author*

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A B S T R A C T

Experiments of field and laboratory were carried out to evaluate the effect of different decontamination processes on reduction of chlorpyrifos and cypermethrin residues in cauliflower curds like washing, cooking, washing plus cooking and dipping in chemical solutions after application of ready-mix formulation Cannon 55EC (chlorpyrifos 50% + cypermethrin 5%) on the crop. Ready-mix formulation Cannon 55EC was applied twice at the rate of 1ml/L at 15 days interval on cauliflower crop. Cauliflower curds were collected at 0 (2 hours), 3 and 7 days interval after the last spray and subjected to decontamination processes. Washing of zero day contaminated curd samples provided 37.41% relief from chlorpyrifos residues and 40.10% relief from cypermethrin residues. Cooking degraded chlorpyrifos residues up to 36.58-58.95% and cypermethrin residues by 45.45-66.31%. Washing plus cooking removed chlorpyrifos and cypermethrin residues up to 70% as compared to other processes and proved to be the best technique in removing the residues. Washing of curds with 2% NaOH solution reduced the chlorpyrifos residues up to 60.97-69.03%, whereas washing with 0.05% solution of HCl reduced the chlorpyrifos residues up to 58.53-65.06%. Similarly cypermethrin residues were reduced to 62.50-69.20% after treatment with 2% NaOH solution and up to 63.44-66.97% after treatment with 0.05% HCl solution.

Introduction

Vegetables are the inseparable component of Indian cuisine and are consumed throughout the country in different forms and preparations. They are the major source of vitamins and nutrients; hence they fulfil the requirements of our balanced diet (Chandra *et al.,* 2015). Cauliflower (*Brassica oleracea* var. *botrytis* L.) is an important cash crop of Himachal Pradesh which is infested by a large number of insect-pests and diseases (Sharma and Bhalla, 1964; Sharma, 1975; Bhalla and Pawar, 1977). The key pests of cauliflower are diamond back moth, leaf eating caterpillar and aphids (Regupathy *et al.,* 1985; Patel *et al.,* 1999) thus affecting both the quantity and quality of curds. In a desperate bid to save the crop farmers sometime apply the pesticide repeatedly and at higher doses hence the repeated and intensive use of insecticides have lead to the development of resistance in insect pests (Gaganpreet *et al.,* 2017). In Himachal Pradesh pesticides such as chlorpyrifos and

cypermethrin have been used extensively by the farmers to control these major insect-pests of cauliflower both individually and as ready-mix formulations. Since, the effect of pesticide mixtures is considered more toxic than their individual components, extra care should be taken to reduce the health hazards to the consumers (Regupathy *et al.,* 2004). The application of these pesticides near to harvest can leave residues on the curds which may be harmful to the consumers (Banshtu *et al.,* 2015).

Cauliflower is consumed as raw and cooked vegetable; hence chances of carrying pesticide residues to the consumers are more (Raj *et al.,* 1991). Hence Pesticide residues in cauliflower are of major concern to consumers due to their negative health effects. They have been found in both raw and processed fresh produce. There have been various reports suggesting use of different simple household processes in dislodging pesticide residues from food commodities thus making them safe for human consumption (Sharma *et al.,* 1994; Aktar *et al.,* 2009; Chavarri *et al.,* 2005; Dejonckheere *et al.,* 1996; Elkins, 1989; Krol *et al.,* 2000; Schattenberg *et al.,* 1996). Operations such as Washing, peeling, blanching and cooking play a crucial role in the reduction of residues (Elkins, 1989; Kaushik *et al.,* 2009). Each operation has a cumulative effect on the reduction of the pesticides (Geisman *et al.,* 1975).

So in the present scenario it is very important that some pragmatic solution should be developed to tackle this problem of food safety. Food safety is an area of growing concern worldwide on account of its direct bearing on human health. The presence of harmful pesticide residues in food such as cauliflower has caused a great concern among the consumers. Therefore, the present investigations were contemplated with the objective to study the effect of different decontamination processes in curds for the reduction of chlorpyrifos and cypermethrin residues after the application of ready-mix formulation on cauliflower crop in the field. Hence the techniques used in the present study focused on commercial and home processing of cauliflower and they included washing alone, washing with chemicals, cooking and washing followed by cooking.

Materials and Methods

Chemicals and reagents

Ready-mix formulation Cannon 55EC containing 50% chlorpyrifos and 5% cypermethrin was obtained from M/S Nagarjuna Agrichem Ltd. and reagents like acetone, dichloromethane, hexane, toluene, sodium chloride, sodium sulphate anhydrous (AR grade), Celite 545 and Florisil were all procured from M/S Merck Specialities, Mumbai. Activated charcoal decolorizing powder was obtained from M/S Darmstadt, Germany. All common solvents were redistilled in an all-glass apparatus before use.

Field trials

Cauliflower (*Brassica oleracea* var. *botrytis* L.) was raised during 2010 at Entomological Farm, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh following recommended agronomic practices (Anonymous, 2010).

The experiment was conducted in randomized block design (RBD) with three replications for each treatment. The first application of Cannon 55EC @ 1 ml/L of water was made at curd formation stage followed by second application at an interval of 15 days. In control plots, only water was sprayed. Pesticide was sprayed as foliar application in three replications with the help of a knapsack sprayer, fitted with a hollow cone nozzle.

Sampling procedure

Curd samples (1kg) from each replication were collected randomly at 0 (2 hours after spray), 3 and 7 days intervals after last foliar application. The samples from each replication were collected randomly, packed in bags and brought to the laboratory for processing.

Decontamination processes

Samples collected from the field were subjected to different decontamination processes viz. washing, cooking and washing followed by cooking (Patyal *et al.*, 2004).

Washing

Cauliflower curds were washed under running tap water and hand rubbed for 2 minutes.

Cauliflower curds samples were dipped in lukewarm water (50^0C) for 5 minutes and then, placed on filter papers for drying.

Curd samples were dipped in 2% NaCl (w/v) solution for 5 minutes followed by tap water washing.

Curd samples were dipped in 2% lukewarm salt solution (w/v) for 5 minutes followed by water washing.

Curd samples were dipped in 0.05% HCl (v/v) for 5 minutes, followed by water wash.

Curd samples were dipped in 2% (w/v) sodium hydroxide solution for 5 minutes, followed by washing with water.

Cooking

Open pan cooking: Unwashed samples from each replication were chopped and put in an open pan of 1 litre capacity containing 500 ml water and boiled till softness (10-15 minutes).

Steam cooking: Samples were chopped and steamed for 5 minutes in a pressure cooker.

Microwave cooking: Curd samples were kept in microwave for 5 minutes for cooking at 1400 W power output.

Washing followed by cooking

Washing + cooking: Curd sample were first washed by hand rubbing under a stream of running tap water for 2 minutes, followed by boiling in an open pan of 1 litre capacity containing 500 ml water till soft (10-15 minutes).

Washing + steam cooking: Sample were washed under running tap water and steamed for 5 minutes in a pressure cooker.

Washing + microwave cooking: Samples were first washed under the tap water and then, placed in microwave for 5 minutes for cooking at 1400 W power output.

After completing decontamination process, samples were extracted and cleaned up according to the method of Sharma (2007).

Extraction and cleanup

The samples were processed and analyzed at the Pesticide Residue Analysis Laboratory, Department of Entomology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. Processed cauliflower curd samples were homogenised in a domestic mixture.

A representative 100 g homogenised sample was taken up with 200 ml acetone in a 500 ml conical flask and kept for overnight. The extract was filtered through Buchner funnel by fitting a Whatman No. 1 filter paper. An aliquot of 60 ml (30 g equivalent) of sample was transferred to 1 litre separatory flask and

extracted with 200 ml mixture of hexane and dichloromethane (1:1, v/v). The lower aqueous phase was transferred to another 1 litre separatory funnel containing ten millilitre saturated sodium chloride solution and partitioned twice with 100 ml dichloromethane. Lower aqueous phase was discarded and upper organic phase was pooled with first organic fraction. Pooled organic phase was passed through anhydrous sodium sulfate and evaporated to dryness at 45^0C by using vacuum rotary evaporator. Finally, the residues were taken up in 3 ml (1+2) acetone for cleanup. Samples for cypermethrin residues were cleaned up on Florisil column and chlorpyrifos samples were cleaned up on charcoal column.

First sample fraction of 1 ml was diluted with 10 ml of acetone: hexane (1:9) mixture, loaded on the 4 g activated Florisil column overlaid with 2 g sodium sulfate. The column was eluted with 50 ml eluent (50% dichloromethane: 48.5% hexane: 1.5% acetonitrile). Eluant was evaporated to dryness, residues were dissolved in 1 ml n-hexane and injected into gas chromatograph.

Two millilitres of sample fraction was loaded in a charcoal column which was prepared by placing one inch layer of Celite 545, 6 g adsorbent mixture (1:4 w/w Charcoal: Celite 545) and then, overlaid with 2 g sodium sulfate. The column was eluted with 200 ml of 2:1 acetone: dichloromethane mixture. Eluant was evaporated to dryness, residues were dissolved in 2 ml n-hexane and injected 1µl into a gas chromatograph.

Residue estimation

Residues of chlorpyrifos and cypermethrin were estimated by using Gas-Chromatograph (Agilent 6890N) having ECD detector and DB-5 Ultra Performance Capillary column (Cross-linked Methyl Silicon, length 30 m, 0.25 mm internal diameter with 0.25 µm film thickness). Oven temperature was programmed as: 100^0C for 1 minute, 30^0C/minute up to 150^0C, 3^0C/ minute up to 205^0C and finally 260^0C at rate of 10^0C/ minute. Injection port and electron capture detector (ECD) temperature were kept at 250^0C and 300^0C, respectively.

Chlorpyrifos and cypermethrin residues (mg/kg) were determined for each replication and then mean residues were calculated. Per cent relief from residues in each treatment was calculated from the mean residues, by the following equation:

% relief = 100 - (Residue in processed sample (mg/kg) / Residue in unprocessed sample (mg/kg)) X 100)

Validation of analytical method

The analytical method employed to estimate chlorpyrifos residues was validated by spiking the control curd samples at four different concentrations viz., 0.01, 0.05, 0.10 and 0.5 mg/kg whereas, cypermethrin samples were spiked at 0.05, 0.10, 0.50 and 1.0 mg/kg concentrations. Recovery of chlorpyrifos was between 90.00-94.00% with relative standard deviation (RSD) of 0.112-1.030% in fruits and recovery of cypermethrin was between 90.00-92.00% with RSD of 0.062-0.988% (Table 1).

Results and Discussion

Effect of washing

Washing is the most common form of processing which is a preliminary step in both household and commercial preparation. Loosely held residues of several pesticides are removed with reasonable efficiency by varied types of washing processes (Street, 1969). Washing of 0 day sampled cauliflower curds under running tap water provided 37.41%

relief whereas 33.80 and 29.26% relief from chlorpyrifos residues was observed in 3 and 7 day old samples, respectively (Figure 1). Similar observations were recorded after washing of cauliflower curds treated with cypermethrin (Figure 3). Aktar *et al.,* (2010) reported that washing of cabbage head under running tap water removed 27.72-32.48% quinalphos residues which are in accordance with my findings. Similarly, Singh *et al.,* (2004) also found that washing of okra fruits with tap water could remove the residues of cypermethrin to the extent of 36.25-42.76%. The initial diazinon residue level (0.822 ppm) on cucumbers was decreased by 22.3% by washing for 15 seconds rubbing under running water (Cengiz *et al.,* 2006).

Lukewarm water washing of 0 day sampled cauliflower curds provided 41.21% relief whereas 39.29 and 36.58% relief from chlorpyrifos residues was observed in 3 and 7 days old samples, respectively (Fig. 1). Similar observations were recorded after washing of cauliflower curds treated with cypermethrin (Fig. 2) which are in accordance with Kanta *et al.,* (1998) who reported 7-38 per cent reduction of *alpha*-cypermethrin residues by lukewarm water washing of cauliflower curds. Kumari (2008) also reported 32-100 per cent reduction of OP's insecticide residues by lukewarm water of cauliflower.

Chemical washing

Washing of treated cauliflower curds with sodium hydroxide and hydrochloric acid provided a good relief from chlorpyrifos and cypermethrin in comparison to washing with sodium chloride and lukewarm sodium chloride solution. It may be due to hydrolytic property of chlorpyrifos and cypermethrin in strong acids and alkalis (Tomlin, 1995). Sodium hydroxide provided 69.03% and 70.14% relief from chlorpyrifos and cypermethrin, respectively. Dip treatment of cauliflower curds with hydrochloric acid gave 65.06% relief from chlorpyrifos and 69.43% from cypermethrin residues. The present findings are in agreement with Patyal *et al.,* (2004) who found that washing of treated apple fruits with 2% (w/v) NaOH and 0.05% (v/v) HCl gave 77.06 and 75.96%, relief respectively from endosulfan residues.

Marshall (1982) also reported that unwashed samples of green beans contained an average of 1.49 ppm of EBDC (Ethylenebis dithiocarbamates) and very low levels of its metabolite ethylenethiourea (ETU). Washing of beans in cold water for 2 min removed 45% of EBDC but did not affect the levels of ETU whereas, the wash with alkaline hypochlorite followed by dipping in dilute sodium sulfite left no detectable residues of EBDC or ETU on the beans.

Dipping of cauliflower curd samples in 2% sodium chloride solution (w/v) reduced chlorpyrifos and cypermethrin residues to 43.03% and 46.21% whereas lukewarm sodium chloride solution reduced residues to 51.18% and 61.13% respectively which is in agreement with the findings of Mukherjee *et al.,* (2006) also observed that dipping of cauliflower curds in 1% brine solution followed by washing reduced the residues by 39.6% while in case of hot 1% brine solution, the reduction was 55.0%.

Cooking

Application of heat to the food commodities is commonly done through ordinary cooking, pressure cooking, microwave cooking, frying, sterilization and canning. The effect of different cooking processing on removal of chlorpyrifos and cypermethrin residues in cauliflower was studied (Figure 1 and 2). In all of the processes, cooking with pressure cooking was found to be more effective than in others.

Fig.1 Per cent relief from chlorpyrifos residues from different decontamination processes (W= Tap water washing, LW= Luke warm, OPC=Open pan cooking, PC=Pressure cooking, MC=Microwave cooking)

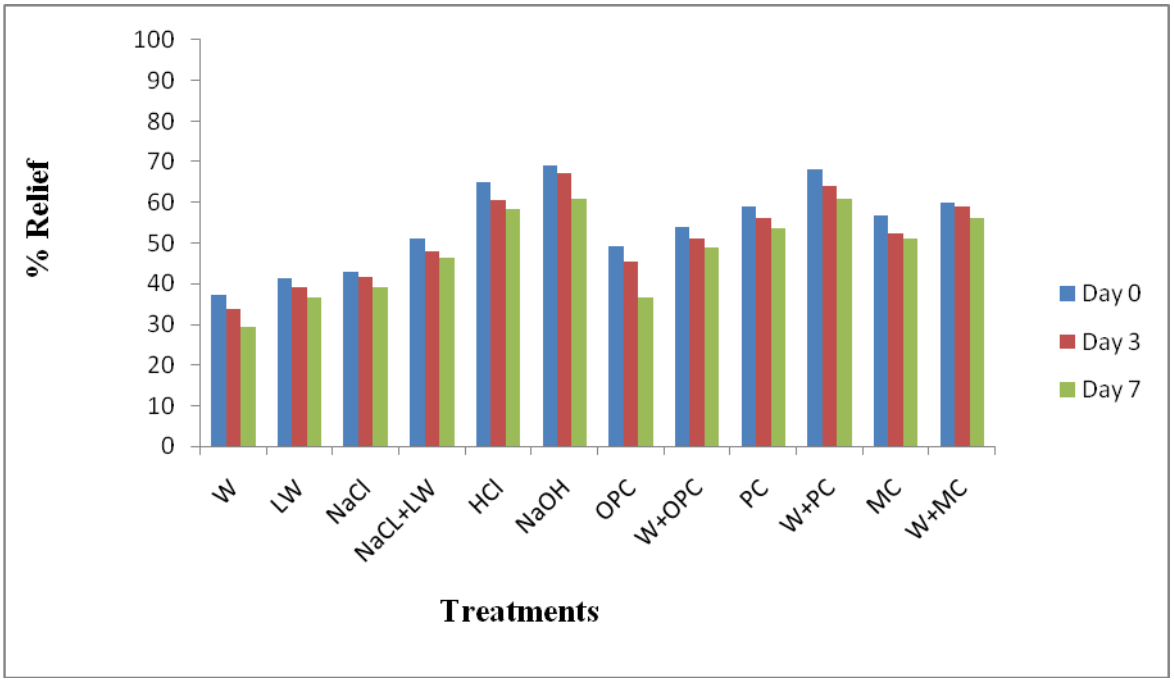

Fig.2 Per cent relief from cypermethrin residues from different decontamination processes (W= Tap water washing, LW= Luke warm, OPC=Open pan cooking, PC=Pressure cooking, MC=Microwave cooking)

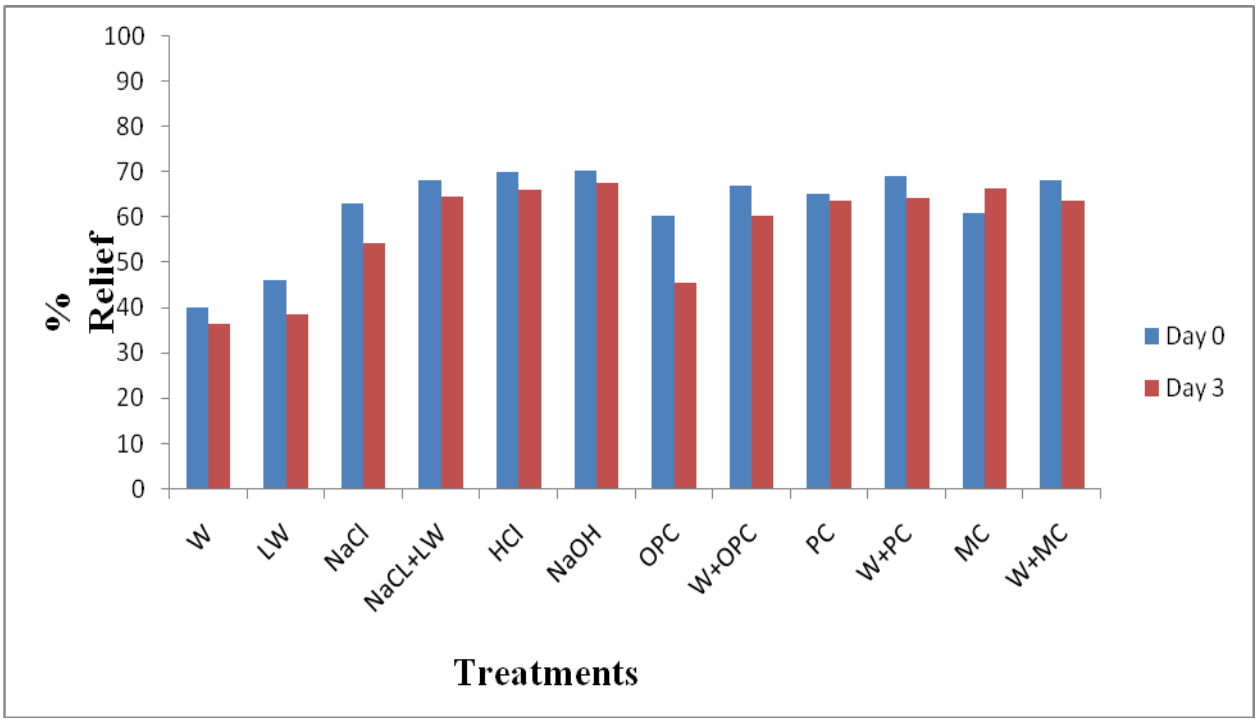

Table.1 Recovery of chlorpyrifos and cypermethrin from cauliflower curds

Insecticides	Cauliflower curds		
	Fortification level, (mg/kg)	Mean recovery (%)	Relative standard deviation (%RSD)
Chlorpyrifos	0.01	90.00	1.030
	0.05	92.00	0.987
	0.10	93.00	0.858
	0.50	91.00	0.178
Cypermethrin	0.05	90.00	0.988
	0.10	90.00	0.525
	0.50	90.00	0.160
	1.00	92.00	0.062

Pressure cooking reduced the residues up to 58.95%. These results are in accordance with the findings of Muthukumar *et al.,* (2010) who also reported that pressure cooking was the most effective in reducing both α- and β-endosulfan by 64.59% and 61.60% as compared to boiling and microwave cooking.

Cooking of cauliflower curds in open pan or under pressure or in the microwave resulted in 50-60% relief from chlorpyrifos and cypermethrin residues. The findings are in agreement with Dikshit (2001) who observed that process of steaming dislodged the cypermethrin residues by 63-74% on stored pulses treated at 3 and 5 mg/kg levels. The disappearance of pesticide residues from boiling extract could be due to decomposition by the effect of heat, the stronger adsorption of pesticide onto plant tissues and or/the poor solubility of pesticides in water (Abou and Abou 2001; Ali, 1983). Walia *et al.,* (2010) reported that microwave cooking reduced cypermethrin residues to the extent of 40.89 per cent in brinjal sprayed at 0.001 per cent concentration. Hence, processes involving heat can increase volatilization, hydrolysis or other chemical degradation and thus, reduce residue levels (Holland *et al.,* 1994).

Washing followed by cooking

Washing is generally the first step in various types of treatments which are given to food commodities in combinations like washing followed by cooking, washing and drying, washing and peeling and washing, peeling and juicing to allow for effective decontamination from pesticides (Kaushik *et al.,* 2009).

Washing of cauliflower curds followed by cooking lead to more than 65% removal of chlorpyrifos and cypermethrin residues (Figure 1 and 2). Similarly, Mukherjee *et al.,* 2006 also reported that washing of cauliflower heads under running tap water removed 27.9% chlorpyrifos residues, cooking reduced residues to 41.4% and washing + cooking further reduced residues to 66.7%. Aktar *et al.,* (2010) also reported that washing plus cooking of cabbage heads reduce more quinalphos residues (66.45-68.19%) in comparison to washing alone (41.30-45.20%).

A critical analysis of whole decontamination data revealed that the washing plus pressure cooking removed much higher residues from

contaminated curds as compared to the simple washings. Although, sodium hydroxide and hydrochloric acid treatments were superior over all other decontamination processes but such treatments can be used in the industries where large quantity of vegetables are processed for decontamination. Washing of vegetables with water followed by pressure cooking removed maximum residues upto 70% as compared to the other processes and proved good household practice.

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