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RESEARCH PAPER

Effect of Decontamination Processing on Profenofos and Cypermethrin Residues in Tomato Fruits

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Abstract

Laboratory experiments were carried out to evaluate the effect of different tomato fruit decontamination processes like washing, cooking, washing plus cooking and dipping in chemical solutions on reduction of profenofos and cypermethrin residues after application of ready-mix formulation Roket 44EC (profenofos 40% +cypermethrin 4%) on the crop. Ready-mix formulation Roket 44EC was applied twice at the rate of 1ml/L at 15 days interval. Tomato fruits were collected at 0 (2 hours) and 3 days interval after the last spray and subjected to decontamination processes. Washing of zero day contaminated fruit samples provided 32.95-72.08% relief from profenofos residues and 38.12-75.17% relief from cypermethrin residues. Cooking degraded profenofos residues up to 44.74-65.77% and cypermethrin residues by 61.00-66.02%. Washing plus cooking was found to be the best technique in removing the residues. Washing of fruits in solutions like NaOH (2%) and HCl (0.05%) has also shown a good relief from profenofos and cypermethrin residues.

Keywords: Tomato, processing, profenofos, cypermethrin, washing, cooking, residues

Vegetables are the inseparable components of Indian cuisine and are consumed throughout the country in different forms and preparations. They form the bulk and are the major source of vitamins and nutrients, hence fulfilling the requirements of our balanced diet (Chandra *et al*. 2015). Among the vegetables, tomato gives better return to the farmers but it is attacked by many insect pests like tomato fruit borer, mites, leaf miner, aphids, white flies etc. affecting both quantity and quality of fruits. Farmers spray insecticides to control these insect pests but profenofos and cypermethrin are very effective in controlling these pests. Repeated and intensive use of insecticides leads to the development of resistance in insect pests. The development of resistance and resurgence has limited the application of single insecticide and resort to tank mixtures. The insecticide mixtures can be classified into two major groups, the tank mixtures and prepacked mixtures (ready-mix formulations). The tank mixtures are prepared in the field directly by farmers little time before spraying. This type of mixtures arises from intuition of farmers and amount of diluents in the tank mixture increases when the two formulations are mixed in the field. Hence, crop receives an overdose of diluents and there may be negative interaction with the toxicants when there is no compatibility. These problems have been overcome by allowing reputed companies to formulate and register ready-mix formulations by the Central Insecticide Board Registration Committee. The ready-mix formulations were reported to be very effective in managing the pests in different crop ecosystems (Mallikarjunappa *et al*. 2002). They were found to be better than the efficacy of the component chemicals when applied alone (Dharumarajan and Dikshit, 2010). Since, the effect of pesticide mixtures is considered more toxic than their individual components, extra care should be taken to reduce the health hazards (Regupathy *et al*. 2004). Tomato fruits are picked frequently at short intervals and consumed, as cooked or raw. The application of pesticides near to harvest can leave residues on the fruits which may be harmful to the consumers.

Food safety is an area of growing worldwide concern on account of its direct bearing on human health. The presence of harmful pesticide residues in food has caused a great concern among the consumers. Hence, world over to tackle food safety issues, the practice of organic farming is being propagated. However, due to several reasons, diffusion and acceptance of this approach in developing countries has been very slow. Therefore, it is important in the transient phase that some pragmatic solution should be developed to tackle this problem of food safety. Pesticide residues in vegetables are of major concern to consumers due to their negative health effects. They have been found in both raw and processed fresh produce. However, food processing techniques have been found to significantly reduce the pesticide residues in fruits and vegetables in several studies (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 present (Geisman *et al*. 1975). The techniques used in the study focused on commercial or home processing of vegetables and they included washing alone, washing with chemicals, cooking, washing followed by cooking.

Therefore, the present investigations were carried out to study the effect of different fruit decontamination processes on profenofos and cypermethrin residues after the application of ready-mix formulation on the tomato crop in the field.

MATERIALS AND METHODS

Chemicals and reagents

Ready-mix formulation Roket 44EC containing 40% profenofos and 4% cypermethrin was obtained from M/S P I Industries Ltd. and reagents like acetone, dichloromethane, hexane, toluene, sodium chloride, sodium sulfate 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. The suitability of the solvents and other chemicals was ensured by running reagent blanks, before actual analysis.

Field trials

Tomato (var. Him Sohna) was raised during 2009 at Entomological Farm, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh following recommended agronomic practices (Anonymous, 2009). The experiment was conducted in randomized block design (RBD) with three replications for each treatment.

The first application of Roket 44EC (profenofos 40%+cypermethrin 4%) @ 1 ml/L of water was made at fruit 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

Fruit samples (1kg) from each replication were collected randomly at 0 (2 hours after spray) and 3 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.

(A) Washing

1. *Washing with running tap water:* Tomato fruits

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

2. *Lukewarm water washing:* Tomato fruit samples were dipped in lukewarm water (50°C) for 5 minutes and then, placed on filter papers for drying.
3. Washing with sodium chloride solution: Fruit samples were dipped in 2% NaCl (w/v) solution for 5 minutes followed by tap water washing.
4. *Washing with lukewarm sodium chloride solution:* Fruit samples were dipped in 2% lukewarm salt solution (w/v) for 5 minutes followed by water washing.
5. *Washing with hydrochloric acid solution:* Fruit samples were dipped in 0.05% HCl (v/v) for 5 minutes, followed by water wash.
6. *Washing with sodium hydroxide solution:* Fruit samples were dipped in 2% (w/v) sodium hydroxide solution for 5 minutes, followed by washing with water.

(B) Cooking

1. *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).
2. *Steam cooking:* Samples were chopped and steamed for 5 minutes in a pressure cooker.
3. *Microwave cooking:* Fruit samples were kept in microwave for 5 minutes for cooking at 1400 W power output.

(C) Washing followed by cooking

1. *Washing+cooking:* Fruit 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).
2. *Washing+steam cooking:* Sample were washed under running tap water and steamed for 5 minutes in a pressure cooker.
3. *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 tomato fruit 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°C 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 profenofos 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 profenofos 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˚C for 1 minute, 30˚C/minute up to 150˚C, 3˚C/ minute up to 205˚C and finally 260˚C at rate of 10˚C/ minute. Injection port and electron capture detector (ECD) temperature were kept at 250˚C and 300˚C, respectively.

Profenofos 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 =

$$100 - \left(\frac{\text{Residue in processed sample (mg/kg)}}{\text{Residue in unprocessed sample (mg/kg)}} \times 100 \right)$$

Validation of analytical method

The analytical method employed to estimate profenofos and cypermethrin residues was validated by spiking the control fruit samples at five different concentrations viz., 0.05, 0.10, 0.20, 0.5 and 1.0 mg/kg. Recovery of profenofos was between 86.00-93.00% with relative standard deviation (RSD) of 0.034-0.870% in fruits and recovery of cypermethrin was between 88.00-90.00% with RSD of 0.034-0.738% (Table 1).

Table 1: Recovery of profenofos and cypermethrin from tomato fruits

Insecticides	Tomato fruits		
	Fortification level, (mg/kg)	Mean recovery (%)	Relative standard deviation (%RSD)
Profenofos	0.05	88.00	0.870
	0.10	86.00	0.349
	0.20	89.00	0.086
	0.50	91.00	0.034
	1.00	93.00	0.038
Cypermethrin	0.05	88.00	0.738
	0.10	88.00	0.286
	0.20	89.00	0.202
	0.50	89.00	0.147
	1.00	90.00	0.034

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 tomato fruits under running tap water provided 32.95% relief whereas 43.72% relief from profenofos residues was observed in 3 days old samples (Fig. 1). Similar observations were recorded after washing of tomato fruits treated with cypermethrin (Fig. 2). Aktar *et al*. (2010) reported that washing of cabbage head under running tap water removed 27.72-32.48% quinalphos residues.

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%. Bitter gourds treated with endosulfan sprays received initial deposits of 18.97 ppm and 26.01 ppm which were respectively removed to extent of 59.05% and 42.66% by 30 seconds of washing (Nath and Agnihotri, 1984). The removal of pesticide residues by washing has been found to depend on the age of the chemical

(Guardia *et al*. 2007). 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).

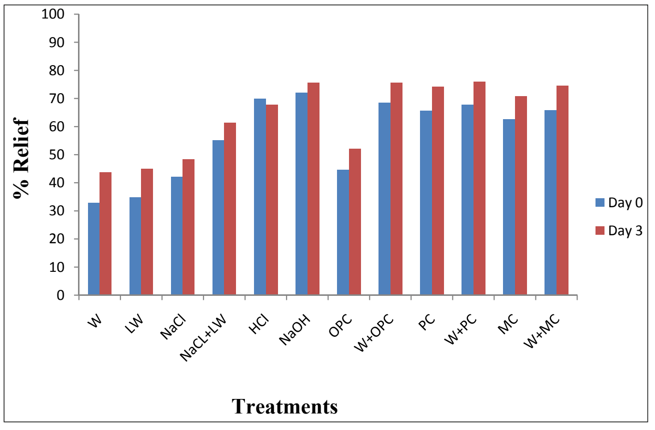

Fig. 1: Per cent relief from profenofos residues from different decontamination processes (W = Tap water washing, LW = Luke warm, OPC = Open pan cooking, PC = Pressure cooking, MC = Microwave cooking)

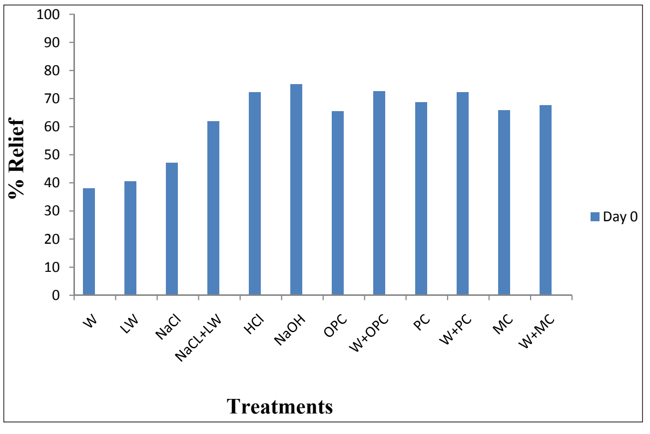

Fig. 2: Per cent relief from cypermethrin residues from different decontamination processes (W = Tap water washing, LW = Luke warm, OPC = Open pan cooking, PC = Pressure cooking, MC = Microwave cooking)

Lukewarm water washing of 0 day sampled tomato fruits provided 34.92% relief whereas 45.08% relief from profenofos residues was observed in 3 days old samples (Fig. 1). Similar observations were recorded after washing of tomato fruits treated with cypermethrin (Fig. 2). Kanta *et al*. (1998) reported 7-38 per cent reduction of *alpha*-cypermethrin residues by lukewarm water washing of cauliflower curds. Kumari (2008) reported 32-100 per cent reduction of OP's insecticide residues by lukewarm water of cauliflower.

Chemical washing

Washing of treated tomato fruits with sodium hydroxide and hydrochloric acid provided a good relief from profenofos and cypermethrin in comparison to washing with sodium chloride and lukewarm sodium chloride solution. It may be due to hydrolytic property of profenofos and cypermethrin in strong acids and alkalies (Tomlin, 1995). Sodium hydroxide provided 72.08% and 75.17% relief from profenofos and cypermethrin, respectively. Dip treatment of tomato fruits with hydrochloric acid gave 69.98% relief from profenofos and 72.30% 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%, respectively relief 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.

Tomato contaminated at level of 1 ppm upon washing with different levels of acetic acid solution gave 51.3%, 47.0%, 33.7%, 91.5%, 86.0% and 93.7% loss in HCB, lindane, p,p-DDT, dimethoate, profenofos and pirimiphos-methyl, respectively. Sodium chloride washing came next in importance to washing by acetic acid solutions, giving 42.9%, 46.1%, 27.2%, 90.8%, 82.4% and 91.4% loss in the same pesticides, respectively (at 10% NaCl). The trends of the data indicated that the loss of different pesticides depends on the levels of acetic acid and NaCl solutions (2%, 4%, 6%, 8%, and 10%). On the other hand, washing by tap water proved the least effective, showing 9.62%, 15.3%, 9.17%, 18.8%, 22.7% and 16.2% loss of HCB, lindane, p,p-DDT, dimethoate, profenofos, and pirimiphos-methyl, respectively (Abou, 1999). Washing of treated fruits with sodium bicarbonate and sodium lauryl sulphate solutions reduced the endosulfan residues to 56.09-71.15% and 61.20-70.03%, respectively (Patyal *et al*. 2004). Mukherjee *et al*. (2006) 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 profenofos and cypermethrin residues in tomato was studied (Fig. 1, 2). In all of the processes, washing with pressure cooking was found to be more effective than washing in others. Washing plus pressure cooking reduced the residues up to 67.74%. Muthukumar *et al*. (2010) 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 tomato fruits in open pan or under pressure or in the microwave resulted in 50-70% relief from profenofos and cypermethrin residues. The total residues reported to be removed by cooking alone were 56.7% and 69.7% from maize grains and 64.2% and 75% from beans. The initial level of residues was 2.79 and 4.10 ppm, respectively, on maize grains and beans after 12 months of storage. Though malathion and its polar metabolites, malathion-a and malathion b-monocarboxylic acids were completely eliminated by boiling, malaoxon was still detected in quite high quantities in the solvent extracts of cooked beans and maize (Lalah and Wandiga, 2002). 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). Microwave cooking of rice and beans containing trifluralin, chlorpyrifos, decamethrin, cypermethrin and dichlorvos residues at powers of 500W and 800W for 15–45 min resulted in elimination of 92% to 99% pesticides residues (Castro *et al*. 2003). Blanching and frying of egg plant for 5 min completely removed the profenofos residues which were initially present at level of 0.27 ppm (Radwan *et al*. 2005). 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 tomato fruits followed by cooking leads to more than 65% removal of profenofos and cypermethrin residues (Fig. 1, 2). Washing the apples followed by cooking (including processing apple to sauce) reduced the amount of residue by 98%. The total amount of residue on the control unwashed fruit was determined to be 0.67 ppm (Ong *et al*. 1996). Washing and steaming of chickpea grains completely removed the deltamethrin residues from an initial level of residues of 0.051 ppm (Lal and Dikshit, 2001). Washing of cauliflower heads under running tap water removed 27.9% chlorpyrifos residues, cooking reduced residues to 41.4% and washing+cooking further residues reduced to 66.7% (Mukherjee *et al*. 2006). 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%).

CONCLUSION

A critical analysis of whole decontamination data revealed that the washing plus pressure cooking removed much higher residues from contaminated fruits 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 or for other purposes like NaOH is used as lye. HCl is used to remove the adhered particles from fruit skin. Sodium hydroxide and hydrochloric acid treatments are not feasible at household level. However, all these processes substantially reduce the residues of insecticides in vegetables.

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