

Persistence and Effect of Processing on Reduction of Chlorpyrifos Residues in Okra Fruits

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Abstract Residue levels of chlorpyrifos were determined in unprocessed and processed okra fruits to evaluate the effect of different processes (washing and washing followed by boiling/cooking) on reduction of residues of this pesticide in okra. The study was carried out on okra crop (Variety, Varsha Uphar) in research farm of Chaudhary Charan Singh, Haryana Agricultural University, Hisar with application of chlorpyrifos (Radar 20 EC) at 200 g a.i./ha and 400g a.i./ha (Single Dose, T_1) and 400 g a.i./ha (Double Dose, T_2). Samples of okra fruits were collected on 0, 1, 3, 5, 7, 10, 15 days and at harvest after treatment. Residues were estimated by GC-ECD system and reached BDL of 0.010 mg kg⁻¹ on 7th and 15th day in case of single and double dose, respectively. The residues dissipated with half-life period of 3.15 days at lower dose and 3.46 days at higher dose following biphasic first order kinetics. Processing was found very effective in reducing the levels of chlorpyrifos residues in okra fruits. Maximum reduction (64–77%) was observed by washing + boiling followed by washing (13–35%).

Keywords Chlorpyrifos · Residues · Household processing · Okra · Half-life period

Good knowledge of the pesticides fate in agriculture is necessary to properly assess human exposure and the

environmental impact of these contaminants. Vegetables are an essential component of human diet. Besides providing a variety and bulk to the diet, they are good source of vitamins that are essential for human health (Gupta et al. 1988). Among vegetables, okra (*Abelmoschus Esculentus*) popularly known as lady's finger is an herbaceous hairy annual plant of Malvaceae family, cultivated during spring summer (March–June) and rainy (July–September) seasons in India. Total production of okra in India is 3,683,900 tons in which Haryana's share is 93,700.00 (2.54%) tons (Anonymous 2006). Okra has very high nutritional value and is subjected to ravage by a wide array of insect-pests throughout its growth from germination until harvest. Over 37 insects-pests have been recorded causing damage to this crop (Nayyar et al. 1976). Chlorpyrifos [O, O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] is an organophosphorus broad spectrum insecticidal active ingredient registered for application to more than 40 different food commodities including okra. It is a stable compound in neutral and acidic conditions. It kills insects by direct contact or ingestion and by disrupting the normal functioning of nervous systems. It is affective against both sucking and chewing insects and has been widely used to control pests of various vegetables. It is non-systemic, fairly persistent (Anonymous 2000) but almost insoluble in water (2 mg L⁻¹). Cholinesterase inhibition is the mode of action of chlorpyrifos and is the cause of potential toxicity in human (Oliver et al. 2000). Use of pesticides by farmers is the only way to sort out the problem of insects/pests and many a time they harvest the crop without observing any waiting period. As a result, considerable quantities of these pesticides that are absorbed by vegetables, reach the human body and results in many health hazards. Scientists and food processors have long been interested in the effect of commercial processing on

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persistence of pesticide residues in food. Byrne and Pinkerton (2004) examined the effects of common household heat processing on various types of produce. The results suggested that residues decreased due to different treatments. Therefore the present investigation was carried out with the objectives to examine the persistence behaviour of chlorpyrifos and effect of processing on reduction of its residues in okra fruits.

Materials and Methods

A field experiment was conducted on okra crop (Variety: Varsha Uphaar) at the research farm of Department of Entomology, Chaudhary Charan Singh, Haryana Agricultural University, Hisar during rainy season 2009. Field trial was laid out in Randomized Block Design (RBD) and replicated three times. Plot size was 3.35×3.35 with net size 3.00×3.00 . Spacing between different plot was 30×60 cm. Number of plants per row was 9 and rows/bed were 5. So, total plants per bed were $9 \times 5 = 45$. Chlorpyrifos (Radar 20EC) was applied on okra crop at 200g a.i./ha and 400g a.i./ha (T_1) and 400g a.i./ha (T_2) along with untreated control at fruiting stage. Each treatment including control was replicated thrice. The formulation was diluted with water and sprayed on okra crop whereas control plots were sprayed with water only. Okra fruit samples were collected at 0 (1 h after spray), 1, 3, 5, 7, 10, 15 days and at harvest after treatment in three replicates. Samples collected from field were analyzed at three stages i.e. raw, after washing and washing followed by boiling/cooking to determine chlorpyrifos residues. The okra samples were divided into three portions, one portion was processed as such second after washing and third one after washing followed by boiling/cooking. Washing was performed by placing okra fruits, in a container and rinsed under normal water for 30 s, with gentle rotation by hand as described by Walter et al. (2000) and blotted dry with a paper towel and divided into two parts. For cooking, in 20 g representative samples of okra 10 mL water was added and boiled till softness of okra pieces.

Extraction and clean-up was performed as per method of Nath et al. (2005) with little modification. Representative 20 g of the finely chopped sample was extracted with 100 mL acetone by shaking on mechanical shaker for 1.5 h. Filtered the extract and partitioned thrice with dichloromethane in separatory funnel after diluting with 50 mL of saturated solution of sodium chloride. The organic layer was concentrated on rotary vacuum evaporator to reduce the volume approximately to 10 mL. Glass column (60 cm $\times$ 22 mm i.d.) was packed compactly with activated charcoal and activated Florisil (1:5w/w). Pre-wetted the column with 40 mL of hexane, loaded the

concentrated extract in the column and eluted with 125 mL of hexane: acetone (8:2 v/v) mixture at flow rate of 4 mL/min., concentrated the eluate on vacuum evaporator followed by gas manifold evaporator. Final volume was made to 2 mL in n-hexane for GC analysis.

The final extracts were analyzed on Shimadzu 2010 gas chromatograph (GLC) equipped with capillary column, HP-I (30 m $\times$ 0.32 mm $\times$ 0.25 μ m film thickness of diphenyl/95% dimethyl polysiloxane) and electron capture detector (ECD). The operating parameters of the instrument were: Oven temperatures ($^{\circ}$ C) 150 (5 min) $\rightarrow$ 8 $^{\circ}$ C min $^{-1}$ $\rightarrow$ 190 (2 min) $\rightarrow$ 15 $^{\circ}$ C min $^{-1}$ $\rightarrow$ 280 $^{\circ}$ (10 min), injection port 280 $^{\circ}$ C and detector 300 $^{\circ}$ C. Flow rate of nitrogen (carrier gas) was 60 mL/min, through column was 2 mL/min and split ratio 1:10. Under these operating conditions the retention time of chlorpyrifos was found to be 13.98 min.

The control samples of okra fruits were spiked at 0.10 and 0.25 mg kg $^{-1}$, and processed by following the methodology as described above. The results revealed that percent mean $\pm$ SD recoveries for okra samples at both these levels were 88.30 ± 1.021 and 92.10 ± 0.813 , respectively (Table 1). The results have been reported as such without applying any correction factors. The minimum limits of detection (LOD) were 0.005 mg kg $^{-1}$ and limit of quantification (LOQ) for chlorpyrifos in okra fruits were found to be 0.010 mg kg $^{-1}$.

Results and Discussion

The average initial deposits of chlorpyrifos at single and double dose were observed to be 0.067 and 0.129 mg kg $^{-1}$, respectively (Table 2). Residues decreased very fast and on first day reached to 0.023 and 0.047 mg kg $^{-1}$ showing thereby 65.67 and 63.56% dissipation in single and double dose, respectively. Residues on 7th and 15th day of application reached below detection limit (BDL) of 0.010 mg kg $^{-1}$ in single and double dose, respectively. In both the doses, residues of chlorpyrifos in ready-mix and individual were below MRL value of 0.2 mg kg $^{-1}$ on 0 day (PFA, 1955). Residue data were subjected to statistical analysis for computation of regression equations, half-life ($t_{1/2}$) values and percent degradation. The residues

Table 1 Recovery of chlorpyrifos from okra fruits

Fortification levels (mg kg $^{-1}$)	Average* recovery (%) $\pm$ SD
0.10	88.30 ± 1.021
0.25	92.10 ± 0.813

* Average of three replicates

Table 2 Persistence/dissipation of chlorpyrifos in okra fruits

Days after treatment	Single dose (200 g a.i. ha ⁻¹)		Double dose (400 g a.i. ha ⁻¹)		Mean
	Average residues (mg kg ⁻¹) ± SD	% Dissipation	Average residues (mg kg ⁻¹) ± SD	% Dissipation	
0	0.067 ± 0.009	–	0.129 ± 0.007	–	0.098
1	0.023 ± 0.008	65.67	0.047 ± 0.004	63.56	0.035
3	0.019 ± 0.006	71.64	0.042 ± 0.003	67.44	0.031
5	0.016 ± 0.006	76.11	0.033 ± 0.003	74.41	0.025
7	0.010 ± 0.005	85.07	0.023 ± 0.006	82.17	0.017
10	BDL	100	0.013 ± 0.004	89.92	0.007
15	–	–	BDL	100	
At harvest	–		–		
Mean	0.017		0.036		
Correlation coefficient $r = -0.8925$			Correlation coefficient $r = -0.8885$		
Regression equation $y = 0.6392 - 0.0953x$			Regression equation $y = 1.9351 - 0.0868x$		
$t_{1/2} = 3.15$ days			$t_{1/2} = 3.46$ days		

CD ($p \geq 0.05$) for days = 0.0045, for dose = 0.0022, for days x for dose = 0.006

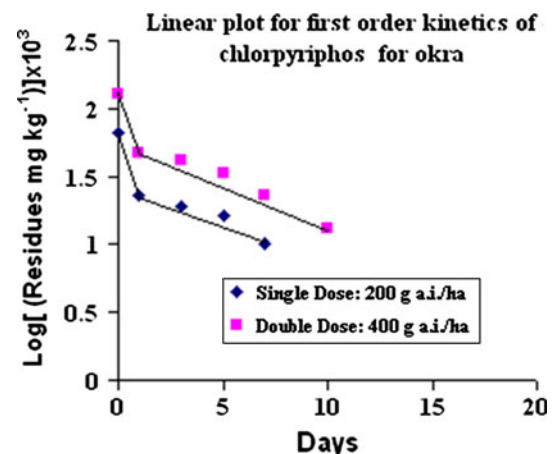
For regression equation, [residues (mg kg⁻¹) × 10³] is taken

* Average ± SD of three replicates; BDL, 0.01 mg kg⁻¹, MRL, 0.2 mg kg⁻¹ (PFA 1955)

dissipated with half-life period of 3.15 days at lower dose and 3.46 days at higher dose following first order kinetics (Fig. 1). Similar type of observation with chlorpyrifos in cabbage has been reported by Patel et al. (1999). The persistence of chlorpyrifos till 9 days in cauliflower has been reported by Raina and Raina (2008) and persistence of quinalphos in cabbage up to 9 days has been reported by Aktar et al. (2010) in which residues dissipated completely within 10 days. Thus present results accord this study.

Effect of Processing

Okra fruits were subjected to processing like washing and washing followed by boiling in order to investigate the reduction of residues. It has been found that washing followed by boiling was found to be more effective than washing in reducing the residues. In this processes, residues could be reduced up to 64.17% in single dose and 69.76% in double dose on 0 day (Table 3). Thereafter reduction of residues was 76.59% on 1st day after application in double dose whereas at single dose residues reached to below detectable limit on 1st day after application. However, by washing, residues were reduced in the range of 18.75–31.34% at lower and 13.04–34.10% at higher dose. From the results it has been concluded that both the processes used in this study were more effective in reducing the residues on 0 day because of the deposition of residues on surface which could be dislodged easily. On the passage of time residues penetrate into fruit and less reduction was observed on successive days. Overall a

**Fig. 1** Dissipation of chlorpyrifos in okra

significant reduction (13.04–34.10%) by washing and (64.17–76.59%) has been observed in present studies. The results are in line with some other recent findings, where washing decreased chlorpyrifos residues in okra by 10–33% by washing and 12–48% by cooking of various vegetables including okra (Randhawa et al. 2007). Reduction of quinalphos residues in cabbage by 27.72–32.48% by washing and 69.02–77.68% by washing followed by cooking have been reported by Aktar et al. (2010). Kumari (2008) reported 50% reduction of organophosphorus residues including chlorpyrifos and 75% by cooking in okra. Holland et al. (1994) reported appreciable reduction in pesticide residues in different commodities by

Table 3 Effect of processing on reduction chlorpyrifos residues in okra fruits

Days after treatment	Single dose (200 g a.i./ha)			Double dose (400 g a.i./ha)		
	Average residues (mg kg ⁻¹)* ± SD	Washing	Washing + cooking	Average residues (mg kg ⁻¹)* ± SD	Washing	Washing + cooking
0	0.067	0.046 ± 0.003 (31.34)	0.024 ± 0.002 (64.17)	0.129	0.085 ± 0.001 (34.10)	0.039 ± 0.002 (69.76)
1	0.023	0.018 ± 0.003 (21.73)	BDL	0.047	0.035 ± 0.002 (25.53)	0.011 ± 0.001 (76.59)
3	0.019	0.015 ± 0.002 (21.05)	–	0.041	0.032 ± 0.002 (21.95)	BDL
5	0.016	0.013 ± 0.002 (18.75)	–	0.033	0.027 ± 0.002 (18.18)	–
7	0.010	–	–	0.023	0.020 ± 0.001 (13.04)	–
10	BDL	–	–	0.013	BDL	–
15	–	–	–	0.010	–	–
At harvest	–	–	–	BDL	–	–

* Average ± SD of three replicates

Figures in *parentheses* are percent reduction of residues in comparison to initial deposits

using different processing methods. Hence the present results are in consistent with the earlier reports.

Conclusion

Thus, a comparison of the overall effects of different household processes indicated that the levels of chlorpyrifos residues can be reduced significantly by washing or by washing + boiling/cooking. The reduction in residue level makes these procedures worthwhile for adoption by the consumers. Residues of chlorpyrifos dissipated below determination level of 0.01 mg kg⁻¹ on 7th and 15th day at single and double dose, respectively. Residues were below MRL value on 10 days in both the doses, hence applied doses were safe additionally washing and washing followed by boiling/cooking was found useful in reducing the residues of chlorpyrifos and therefore these practices should be followed before consumption.

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