



Persistence and effect of decontamination processes on reduction of cypermethrin in okra (*Abelmoschus esculentus*) fruits

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ABSTRACT

Persistence of cypermethrin and decontamination processes on reduction of cypermethrin residues was studied in okra (*Abelmoschus esculentus* L.) fruits following foliar application of formulation at dose of 50 and 100 g a. i./ha in a field at the Research field of CCS HAU, Hisar, India. Samples of okra fruits were collected periodically and residues were estimated by GC-ECD and confirmed by GC-MS. The average initial deposits of cypermethrin were 0.119 and 0.272 mg/kg in the okra fruits at single and double dose, respectively, one hour after the application. Residues of cypermethrin reached equal to below determination level (0.01mg/kg) on 10th in single dose and after 15 days at double dose. Half-life periods for cypermethrin were observed to be 2.74 days and 3.31 days at single and double dose, respectively following first order kinetics. Residues were below maximum residue limit (MRL) value of 0.2 mg/kg on 0 day in single dose and 1 day in double dose. Safe waiting period of 2 days has been suggested for safety sake. Decontamination processes were found very effective in reducing the residues of cypermethrin in okra fruits. Washing reduced the residues in the range of 39–41 %, whereas washing followed by boiling reduced 71 to 79% residues. In soil, residues of cypermethrin reached below detectable level of 0.005 mg/kg after 3rd and 5th days of application at single and double dose, respectively.

Key word: Cypermethrin, Decontamination, Persistence, Okra, Residues

Okra (*Abelmoschus esculentus* L.) also known as *lady's finger* is one of the popular nutritious vegetables of India. Andhra Pradesh with approximately 1184.2 thousand tonnes production from an area of 78.90 thousand ha is a leading okra producing state, followed by West Bengal (862.1 thousand tonnes from 74.00 thousand ha with 11.70 tonnes/ha productivity) (Anonymous 2013). This crop is attacked by large number of insect pests amongst them shoot and fruit borer, *Earias vittella* Fab. and jassid, *Amrasca biguttula biguttula* Ishida cause enormous yield loss (Deen *et al.* 2009). To cope up with insect-pest attack, intensive spray of pesticides are done by the farmers specially at fruiting stage to save the crop from damage, and in turn toxic residues on fruits and soil under crop have been reported (Chandra *et al.* 2014). Thus, these pesticides treated food products (fruits and vegetables) pose threat to consumer's health, hence is one of the serious problem in agriculture. Number of monitoring studies carried out worldwide have reported the residues of pesticides in fruits and vegetables (Essumang *et al.* 2008, Azizi and Hadian 2008, Zawiyah *et al.* 2007, Kumari *et al.* 2006). Among different insecticides, a low dose insecticide, i.e. cypermethrin, is used broadly

to manage several insect pests on vegetables in India (Walia *et al.* 2010). In India, large number of pesticides is registered for use on different crops but now a days there is great concern as well as awareness on the ill effects of pesticides to consumer's health. Thus, the present study was carried out to assess the persistence behaviour and effect of household decontamination processes for reduction residues of cypermethrin on okra fruits at different time intervals.

MATERIALS AND METHODS

Formulation (Cypermethrin 25EC) was procured from local market. The analysis of the formulation in acetone extract with respect to its active ingredient was estimated by gas-liquid chromatography (GLC), no interfering peak except cypermethrin was observed under the retention time of the compound being estimated. Solvents like acetone, dichloromethane and hexane were procured from Merck, Darmstadt, Germany. Sodium chloride (ASC reagent grade $\geq 99.9\%$) was also obtained from Merck, Darmstadt, Germany. Sodium sulfate anhydrous (AR grade) was obtained from SD fine Chemicals Mumbai. All the solvents were redistilled before actual analysis.

Stock solution of cypermethrin having a concentration of 100 $\mu\text{g/ml}$ was prepared in acetone, and for plotting calibration curve, was further used to prepare serial dilution of different concentration, 2.00, 1.50, 1.00, 0.50, 0.25 and 0.10 $\mu\text{g/ml}$ using n-hexane and stored at -4°C .

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To assess the persistence behaviour of cypermethrin in okra fruits, a field study was carried out. For this purpose crop (okra Varsha Uphar) was raised at the Research Farm, Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar, following recommended agronomic practices. There were three replications for each treatment (i.e. control, single and double the application dose) arranged in a randomized block design (RBD) with plot size of 5 × 5 m. Cypermethrin 25EC was applied on okra crop at 50 and 100 g a. i./ha at fruiting stage with the help of Knapsack sprayer. The control plots were sprayed with water only.

The soil under crop was of light texture with low content of organic matter. Other relevant properties of the soil were: EC 2 dS/m; K 10.08, P₂O₅ 15 kg/ha with pH 7.6 and organic carbon 0.67 %.

For residue studies, about 2 kg of marketable size okra fruits were collected randomly from each treated and control plots on 0 (1 h), 1, 3, 5, 7, 10 and 15 days after treatment, mixed thoroughly and brought to the laboratory for further processing. Each sample was further divided into three portions; one portion processed as such by following the method given under section extraction and cleanup. Second part was subjected to household process of washing under tap water for 1 min by gentle rubbing with hand following the method of Walter *et al.* (2000) and the third portion was subjected first to washing under tap water and then to boiling with water till the softening of okra fruits. Second and third portion after washing followed by boiling were processed in a similar manner as of first portion.

Soil samples (1 kg) were collected separately from 10 to 15 sites of each treated plot with the help of a tube auger at a depth of about 0–15 cm with same schedule (0 (1 h), 1, 3, 5, 7, 10 and 15 days after treatment) as of okra fruits. Samples were shade dried, crushed in pestle and mortar and sieved through 2 mm sieve to remove stones/pebbles.

The samples were processed and analyzed at the Pesticide Residue Analysis Laboratory, Department of Entomology, CCSHAU, Hisar. A representative 50g chopped sample was macerated with anhydrous sodium sulphate in Warring blender to make a fine paste and further processes by following the method of Battu *et al.* (2008). The final volume was reconstituted to 2 ml in n-hexane for GC analysis.

Soil samples were processed as per method of Kumari *et al.* (2008). Ground, sieved and dry representative (15 g) of soil sample was mixed thoroughly with 0.3 g activated charcoal, 0.3 g Florisil and 10 g of anhydrous sodium sulphate. The mixture was packed compactly in a glass column (60 cm × 22 mm) in between two layers of anhydrous sodium sulphate. Column was eluted with 125 mL of hexane: acetone (9:1 v/v) mixture at flow rate of 2–3 ml/min. The elute was concentrated on flash evaporator and made up to final volume to 2 mL for GC analysis.

The residues of cypermethrin were determined by gas liquid chromatograph (GLC) model Shimadzu Model 2010 equipped with ECD (Ni⁶³), split ratio (1:10) and fused capillary column (SPB-5) 30 m × 0.32 mm ID, 0.25 µm film

thickness (5% diphenyl and 95% dimethyl) polysiloxane. GC operating conditions were: Carrier gas, N₂ flow, 60 mL/min, split ratio 1:10, injector port temperature 280°C, oven temperature programme was 150°C (5 min) → 8 °C/min 190°C (2 min) → 15°C/min → 280 °C (10 min). Under these operating conditions, the retention times of cypermethrin and its isomers were found to be 20.723, 20.844 and 20.978 min. The residues of cypermethrin in samples were identified and quantified by comparison of retention time and total area of all the isomers of sample chromatograms with that of standards run under identical conditions.

Confirmation of cypermethrin was carried out on gas chromatograph mass detector (GC-MS) model Shimadzu GC-MS-QP 2010 Plus fitted with a 30 m × 0.25 i.d. × / 0.25 µm film Rxi-1ms column and operated in full scan mode (50-500 m/z). Instrument conditions included split less injection, 280°C injector, 300°C transfer line, and 15 °C (5/min) → 8° C/min → 190° C/min (2 min) → 15° C/min → 280°C/min (10 min) and a 28.00 min hold time. Helium was used as a carrier gas with flow rate of 1 mL/min chromatograms are shown in Fig 1 with base peak of 208.6 of cypermethrin (molecular weight 415).

The control samples of okra fruits and soil were spiked at 0.05, 0.01 and 0.005, 0.01 mg/kg respectively, and processed by following the methodology as described above. The mean recoveries of cypermethrin in okra fruits were found to range from 93.20 -95.50 and in soil in the range of 95.00 to 98.50 % (Table 1). Limit of quantification (LOQ) was found to be 0.01 mg/kg for okra fruits and 0.005 mg/kg for soil.

RESULTS AND DISCUSSION

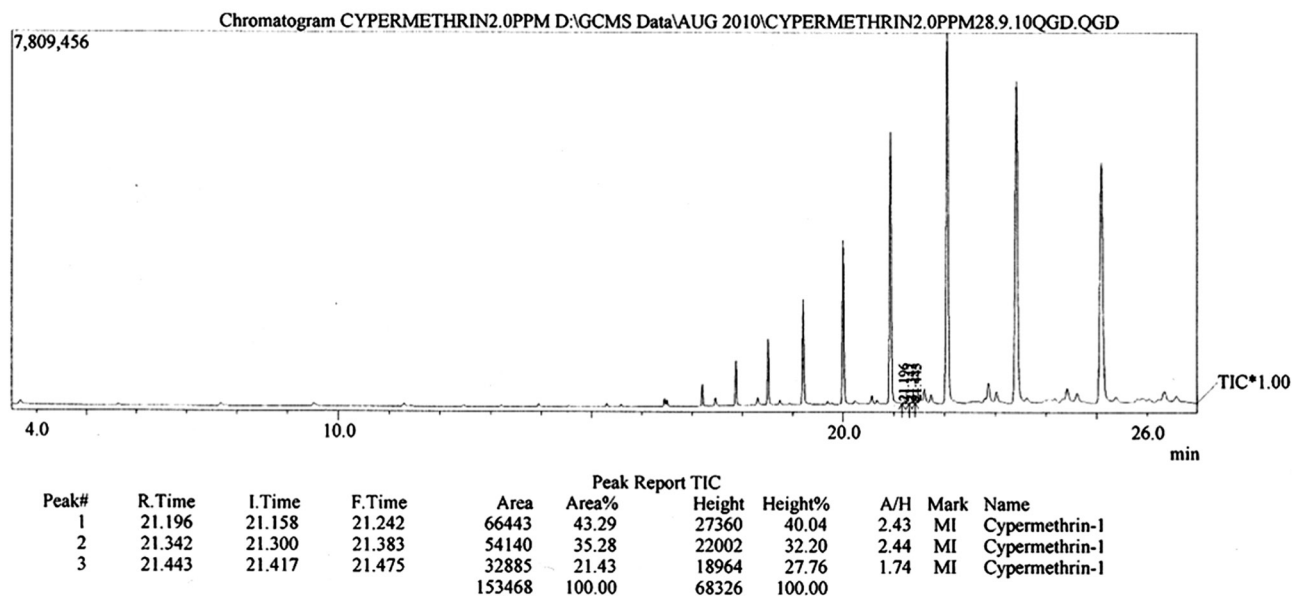
Residues of cypermethrin in okra fruits

Data pertaining to dissipation of cypermethrin residues in okra fruits are presented in Table 2. Average initial deposits of cypermethrin applied with at 50 and 100 g a. i./ha were found to be 0.119 and 0.272 mg/kg, at the respective doses. Residues dissipated with time and reached below detectable level (BDL) of 0.01 mg/kg on 10th day at single dose and BDL after 15 days in double dose. Residues of cypermethrin in single dose were found below maximum residue limit (MRL) of 0.2 mg/kg as set in India for cypermethrin (Sharma 2013) even on 0 day, whereas in case of double dose residues reached below MRL value on 1st day. As the residues are

Table 1 Percent recovery of cypermethrin from okra fruit

Fortification levels (µg/g)	Average* recovery (%) ± SD
	<i>Okra</i>
0.01	93.20 ± 2.01
0.05	95.50 ± 3.32
	<i>Soil</i>
0.005	95.00 ± 0.07
0.01	98.50 ± 1.15

* Average of three replicates



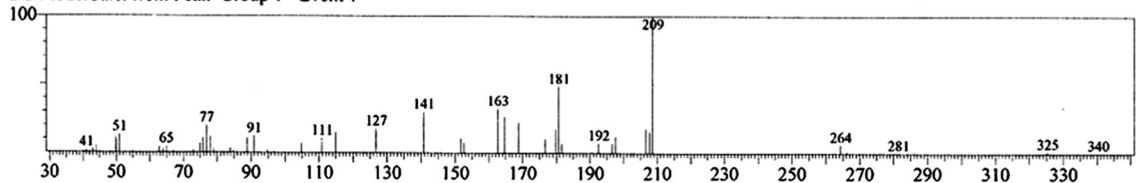
Library

<< Target >>

Line#:1 R.Time:21.200(Scan#:2125) MassPeaks:61

RawMode:Averaged 21.192-21.208(2124-2126) BasePeak:208.60(5085)

BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:320 Library:PESTEI_3.lib

SI:68 Formula:C22H19Cl2NO3 CAS:52315-07-8 MolWeight:415 RetIndex:2858

CompName: Cypermethrin-1 ; (RS)-alpha-cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

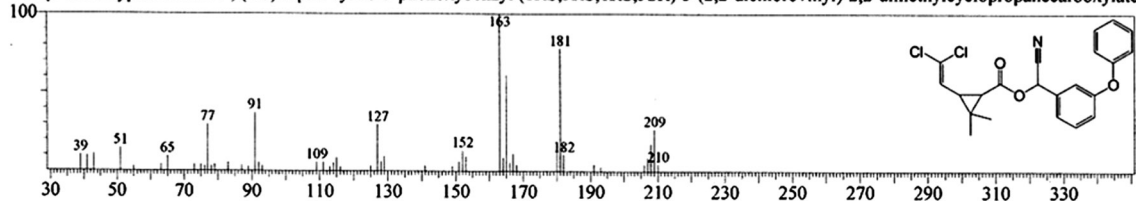


Fig 1 Chromatogram of cypermethrin on GCMS.

below MRL values on zero day in single dose and after 1 day in double dose, thus for safety purpose, safe waiting period of 2 days may be adopted.

Regression equations, half-life values, degradation rate constant and correlation coefficient of cypermethrin shown in Table 2. The half-life values found to be 2.74 days and 3.31 days at single and double doses, respectively. Statistically analysed data using ANOVA showed that irrespective of the duration at single dose, significantly less residues (0.042 mg/kg) were recorded than the double dose (0.097 mg/kg) (CD=-0.0020; P=0.05). With increase in duration, level of residues also decreased significantly (CD=0.0040; P=0.05). Interaction between duration and doses were also significant (CD=0.006; P=0.05).

The present results are in agreement with the earlier reports. Jyot *et al.* (2013) studied the cypermethrin residues on chilli following the application at the rate of 50 and 100

g a.i./ha and observed about 70 % dissipation within a week with half-life period of 2.51 and 2.64 days at single and double the application rates, respectively. Kumari *et al.* (2013) reported of half-life period of 1.58 and 2.18 days following first order kinetics of bifenthrin in tomato after its application at 25 and 50 g a.i./ha. Parmar *et al.* (2012) reported maximum reduction (88.37%) of cypermethrin on 7th day with half-life ($t_{1/2}$) period of 2.59 days following first order kinetics in okra after application at recommended dose. Persistence pattern (behaviour/style) and risk assessment of cypermethrin in chilli was studied by Singh *et al.* (2015) following three applications of cypermethrin at 50 and 100 g a.i./ha at 10 day interval and reported half-life periods of 4.43 and 4.70 days at respective doses. Chandra *et al.* (2014) studied the persistence of different insecticides and reported that the average initial deposits of cypermethrin were in the range of 0.378-0.862 mg/kg. Residues of chlorpyrifos,

Table 2 Residues (mg/kg)* of cypermethrin in okra fruits

Days after treatment	Single dose (50 g a.i./ha)		Double dose (100 g a.i./ha)		Mean
	Average residues \pm SD	% dissipation	Average residues \pm SD	% dissipation	
0	0.119 \pm 0.003		0.272 \pm 0.006		0.196
1	0.090 \pm 0.004	24.36	0.212 \pm 0.003	22.05	0.151
3	0.067 \pm 0.004	43.69	0.147 \pm 0.005	45.95	0.107
5	0.030 \pm 0.002	74.78	0.071 \pm 0.002	73.89	0.051
7	0.020 \pm 0.002	83.19	0.038 \pm 0.003	86.02	0.029
10	0.010 \pm 0.002	91.59	0.024 \pm 0.004	91.17	0.027
15	BDL	100	0.014 \pm 0.002	94.85	0.007
At harvest	-		BDL	100	0.000
Mean	0.042		0.097		
Correlation coefficient	r = -0.9946		Correlation coefficient		r = -0.9774
Regression equation	y=1.0807-0.1096 x		Regression equation		y=1.3726-0.0908 x
t _{1/2}	2.74 days		t _{1/2}		3.31 days

CD (P \geq 0.05) for days = 0.0040, for dose= 0.0020, for days \times for dose= 0.006. For regression equation, [Residues (mg/kg) \times 10³] is taken. * Average \pm SD of three replicates; BDL: 0.01 mg/kg, MRL: 0.2 mg/kg

cypermethrin and monocrotophos reached to BDL showing complete dissipation of each pesticide in 15, 17 and 19 days, respectively when it was applied at 100, 200 and 300 g a. i./ha, on okra. Residues of cypermethrin (43.75 and 87.50 g a. i./ha) and decamethrin (11.20 and 22.40 g a. i./ha) were estimated in brinjal fruits by Kaur *et al.* (2011) and found that residues of cypermethrin and decamethrin reached MRL of 0.2 and 0.05 mg/kg, respectively with half-life values of 1.16 and 1.18 days for cypermethrin and 1.33 and 1.42 days for decamethrin following first order kinetics at, respective doses. Present results are in conformity with the earlier reports.

Effect of decontamination

Decontamination of residues of cypermethrin from contaminated okra fruits include the effect of washing and washing followed by boiling. The data on effect of processing on reduction of residues are shown in Tables 3 and 4. It has been found that washing followed by boiling was found to be more effective than washing alone in reducing the residues. The percent reduction in residues due to washing followed by boiling ranged from 59.66 to 71.64% at single dose and from 64.33 to 78.87% at double dose, whereas reduction of residues by washing ranged from 39.49 to 41.17% in both the doses.

Table 3 Effect of processing on reduction of residues of cypermethrin in okra

Days after treatment	Single dose (50g a.i./ha)			Double dose (100g a.i./ha)		
	Average residues (mg/kg)* \pm SD	Washing	Washing + Cooking	Average residues (mg/kg)* \pm SD	Washing	Washing + Cooking
0	0.119 \pm 0.003	0.072 \pm 0.002 (39.49)	0.058 \pm 0.002 (59.66)	0.272 \pm 0.006	0.160 \pm 0.008 (41.17)	0.097 \pm 0.002 (64.33)
1	0.090 \pm 0.004	0.065 \pm 0.004 (27.77)	0.029 \pm 0.005 (67.77)	0.212 \pm 0.003	0.149 \pm 0.007 (29.71)	0.064 \pm 0.008 (69.81)
3	0.067 \pm 0.004	0.053 \pm 0.002 (20.89)	0.019 \pm 0.005 (71.64)	0.147 \pm 0.005	0.116 \pm 0.004 (21.08)	0.039 \pm 0.005 (73.46)
5	0.030 \pm 0.002	0.025 \pm 0.005 (16.66)	BDL	0.071 \pm 0.002	0.059 \pm 0.003 (16.90)	0.015 \pm 0.002 (78.87)
7	0.020 \pm 0.002	0.017 \pm 0.002 (15.00)		0.038 \pm 0.003	0.032 \pm 0.006 (15.78)	BDL
10	0.010 \pm 0.002	BDL		0.024 \pm 0.004	0.021 \pm 0.002 (12.50)	
15	BDL			0.014 \pm 0.002	0.013 \pm 0.002 (7.14)	
At harvest				BDL		

* Average \pm SD of three replicates; Figures in parentheses is percent reduction of residues in comparison to initial deposits.

Parmar *et al.* (2012) observed cooking as the best process among various culinary processes, as greater level of insecticidal residues was dislodged from okra fruits. Washing followed by cooking of brinjal fruits reduced the residues of cypermethrin to the extent of 41.40% on zero day reported by Walia *et al.* (2010). Chauhan *et al.* (2012) also reported that washing followed by boiling reduced the residues of λ -cyhalothrin in tomato more effectively (74.41-83.87%) as compared to washing (37.50-40.11%) alone. In a study conducted by Kaur *et al.* (2011), washing followed by boiling was found more effective as compared to washing alone where reduction (31-42%) and (26-37%) was observed by washing followed by boiling/cooking for cypermethrin and decamethrin, respectively. Residue levels of bifenthrin were determined in unprocessed and processed okra fruits by Kumari *et al.* (2013) to evaluate the effect of different household processes on reduction of residues at 25 g a. i./ha and 50 g a. i./ha. Maximum reduction (64.58 to 68.42%) was observed by washing + boiling while 36.71 to 40.00 % by washing alone. Hence, the present results corroborate the earlier findings.

Residues in soil

Though cypermethrin was not directly applied to the soil but the samples were collected periodically following the similar schedule as of okra fruits. Residue data and percent dissipation of cypermethrin in soil under field conditions at two doses are presented in Table 5. The average initial deposits estimated in soil were found to be 0.008 and 0.012 mg/kg, which dissipated by 25.00 and 41.66 % after

1 day at single and double doses, respectively, and reached below detectable level (BDL) of 0.001 mg/kg after 3rd day in single dose and after 5th day in double dose. The half-life periods were calculated to be 4.71 and 4.53 days at single and double dose following the first order kinetics. Quite similar results are reported by Jyot *et al.* (2013) where residues of cypermethrin were found to be <0.01 mg/kg at the single and double dosages collected 15 days after the last spray. Following the application of cypermethrin on tomato crop at recommended dose, no residues were detected in soil even on 0 day (Gupta *et al.* 2011). The average initial

Table 5 Persistence of cypermethrin in soil under okra crop

Days after treatment	Residues* (mg/kg)			
	T ₁ (50 g a.i./ha)		T ₂ (100 g a.i./ha)	
	Average \pm SD	% dissipation	Average \pm SD	% dissipation
0	0.008 \pm 0.002		0.012 \pm 0.004	
1	0.006 \pm 0.001	25	0.007 \pm 0.002	41.66
3	0.005 \pm 0.001	37.50	0.006 \pm 0.002	50.00
5	BDL		0.005 \pm 0.002	58.33
7			BDL	
	t _{1/2} = 4.71days		t _{1/2} = 4.53days	

* Average \pm SD of three replicates; BDL: 0.005 mg/kg

deposits estimated in soil under tomato crop were found to be 0.026 and 0.058 mg/kg, respectively, following application of bifenthrin at 25 and 50 g a. i./ha. The residues reached BDL after 5th and 7th day in case of single and double dose, respectively (Chauhan *et al.* 2012).

Conclusion

Residues of cypermethrin in okra fruits were below MRL value of 0.2 mg/kg on zero day in single dose and 1 day in double dose. Additionally decontamination processes indicated that the levels of cypermethrin residues can be reduced significantly by washing and washing followed by boiling. Washing followed by boiling was found more effective than washing alone. Reduction of residues reveals that okra fruits may be safe to consumers after adopting the household processes. Moreover, adoption of 2 days safe waiting period further ensure consumers' safety.

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