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Residual and decontamination study on reduction of pre-mix formulation action-505 EC in Okra

Samriti and Reena Chauhan

Abstract
To check the residue and decontamination status in okra crop treated with pre-mix insecticide [5% cypermethrin + 50% Chlorpyriphos]. Samples were gathered at regular interval after spray of insecticide at 275 g a.i.ha^{-1} (Suggested dose) and 550 g a.i.ha^{-1} (double the suggested dose) from the field of entomology. Residues were analysed by GC-ECD and confirmed by GC-MS. Residue half-life values were 2.74 days and 3.07 days at suggested dose and double the suggested dose for cypermethrin in pre-mix while were 2.16 days and 3.27 days for chlorpyriphos in pre-mix formulation. In household units boiling after washing found much effective in reducing the residues.

Keywords: Pre-mix, decontamination, okra, persistence, residues

Introduction

Okra (*Abelmoschus esculentus* L.) also known as *"lady's finger"* is one of the popular nutritious vegetables of India. It is an important source of vitamins, calcium, potassium, folic acid, carbohydrates and other minerals, which are often lacking in the diet of developing countries. India ranks first in the world with 5,784.0 thousand tonnes (72% of the total world production) of okra. Andhra Pradesh is the leading okra producing state which has production of around 1184.2 thousand tons from an area of 78.90 thousand ha, with a productivity of 15 tons/ha. It is followed by West Bengal (862.1 thousand tons from 74.00 thousand ha with 11.70 tons/ha productivity) [1]. The crop is prone to attack by large number of insect pests among which okra shoot and fruit borer, *Earias vittella* Fab. and jassid, *Amrasca biguttula, biguttula ishida* cause enormous yield loss [2]. From germination to harvesting, the crop is attacked by different insects and pests resulting in low yield production. To protect the crop from damage, intensive spray of insecticides is done by the farmers at fruiting stage, which may leave toxic residues on fruits and soil under crop, which is hazardous to the consumer's health [3]. Consumption of these pesticide treated edible products become unsafe due to the presence of their residues. Numbers of monitoring studies carried out worldwide have reported the residues of pesticides in fruits and vegetables [4-7]. Development of pest resistance to existing pesticides is another problem faced by the farmers [8]. To combat this problem use of pesticide mixture is a promising option. In India, a number of ready pre-mix formulations containing mixture of an organophosphate and a synthetic pyrethroid are registered for use on various crops [10]. Among the various insecticides, cypermethrin, [(RS)-α-cyano-3-phenoxybenzyl-(1-RS)-cis,trans(2,2-dichlorovinyl)-2,2- dimethylcyclopropanecarboxylate] is a synthetic pyrethroid and chlorpyriphos [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. Now-a-days, various ready -mix formulations are marketed in India such as Rocket 44EC (profenophos 40% + cypermethrin 4%), Spark 36 EC (triazophos 35% + deltamethrin 1%), Decidan 32.8 EC(deltamethrin 0.8% + endosulfan 32%), Polytrin C 44EC (profenofos 40% + cypermethrin 4%), Ducord 17 EC (chlorpyriphos 16% + alpha cypermethrin 1%), Nurelle-D (chlorpyrifos- methyl+ profenofos) 0.0055%. These insecticides, separately or in combination, have been found effective in controlling insect pests of vegetables [11-17].

One such ready pre-mix formulation is Action-505 EC (Chlorpyriphos 50%+Cypermethrin 5%). In India, though a number of pesticides are registered for use on various crops but at present an increasing concern and awareness on the hazards of pesticides to consumers. Thus the present study was carried out to investigate the persistence and effect of decontamination household processes in reduction of residues of cypermethrin and chlorpyriphos of the pre-mix formulation Action 505EC in/ on okra fruits at different time intervals.

Materials and Methods

Chemical and reagents

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 ≥ 99.9%) was also obtained from Merck, Darmstadt, Germany. Sodium sulfate anhydrous (AR grade) was from S.D fine Chemicals Mumbai. All the solvents were redistilled before actual analysis.

Preparation of standard solution

Standard stock solution of cypermethrin and chlorpyriphos having a concentration of 100 ppm was prepared in acetone, further serial dilution of various concentrations 2.00, 1.50, 1.25, 1.00, 0.75, 0.50 and 0.25 ppm were prepared in *n*-hexane for drawing calibration curve and stored at -4 ºC.

Field Study

To check the residue status of pre-mix formulation Action-505 EC (5% cypermethrin + 50% chlorpyriphos) in okra produce, a field research was carried out. For this purpose crop (okra [variety- *Varsha uphar*]) was grown at the field site of the university (CCSHAU) premises by the following proposed agronomic practices. There were three replications for each treatment (i.e. control, suggested and double the suggested dose) arranged in a randomized block design (RBD) with plot size of 5 x 5 m. During the research program, average maximum temperature was 40.6°C, average minimum temperature was 23.7°C, relative humidity 57% and zero rainfall. The soil of research field was sandy loam with organic matter 0.67%, pH 7.6 and EC 2.0 dSm^{-1} [18].

Before treatments, the pre-mix insecticide formulation (cypermethrin and chlopyriphos) were tested for their active ingredient, on the basis of which correct amount of insecticide required for each treatment were calculated. Insecticide formulation was sprayed at suggested (275 g a.i. ha^{-1}) and double the suggested dose (550 g a.i. ha^{-1}) whereas one plot was kept as such and used for collection of control samples.

Sampling

For residue studies, about 2 kg of marketable size okra fruits were collected randomly from each treated and control plots on 0 (1h), 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 [19] 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.

Extraction and clean up

Okra

The samples were processed and analyzed at the Pesticide Residue Analysis Laboratory, Department of Entomology, CCS Haryana Agricultural University, 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 [20]. The final volume was reconstituted to 2 ml in n-hexane for GC analysis.

Estimation

The residues of cypermethrin were determined by gas liquid chromatography (GLC) model Shimadzu Model 2010 equipped with ECD (Ni^{63}), split ratio (1:10) and fused capillary column (SPB-5) 30 m x 0.32 mm I.D., 0.25 µm film thickness (5% diphenyl and 95% dimethyl) polysiloxane. GC operating conditions were: Carrier gas, N_2 flow, 60 mL min^{-1}, split ratio 1:10, injector port temperature 280ºC, oven temperature programme was 150^0C (5 min) →8 ^{0}C min^{-1} 190^0 C (2 min) →15^0C min^{-1} →280 ^{0}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 by GC-MS

Confirmation of cypermethrin and chlorpyriphos was carried out on gas chromatograph mass detector (GC-MS) model Shimadzu GC-MS-QP 2010 Plus fitted with a 30 m x 0.25 i. d. x 0.25 µm film Rxi-1ms column (Restek International, U.S.A.) and operated in full scan mode (50-500 m/z). Instrument conditions included spit less injection, 280^0C injector, 300^0 C transfer line, and 15 ^{0}C (5min) → 8^0 C min^{-1}→ 190^0 C min^{-1} (2 min) → 15^0 C min^{-1} → 280^0C min^{-1} (10 min) and a 28.00 min hold time. Helium was used as a carrier gas with flow rate of 1 mL min^{-1} chromatograms are shown in Fig. 1 and 2 with base peak of 208.6 of cypermethrin (molecular weight 415) and 196.15 of chlorpyriphos (molecular weight 349).

Recovery studies

The control samples of okra fruits and soil were spiked at 0.25 and 0.5 mg kg^{-1} respectively, and processed by following the methodology as described above. Average recoveries of cypermethrin and chlorpyriphos in ready-mix were 90.60 and 93.00 percent for cypermethrin, 89.00 and 92.00 percent for chlorpyriphos (Table 1). Limit of quantification (LOQ) was found to be 0.01 mg kg^{-1} for okra fruits.

Table 1: Percent recovery of cypermethrin and chlorpyriphos in ready-mix from okra fruits

Fortification Levels (µg g^{-1})	Average* Recovery for cypermethrin (%)± SD	Average* Recovery for chlorpyriphos (%)± SD
Okra		
0.10	90.60±3.091	89.00±0.041
0.25	93.00±5.01	92.00±2.79

* Average of three replicates

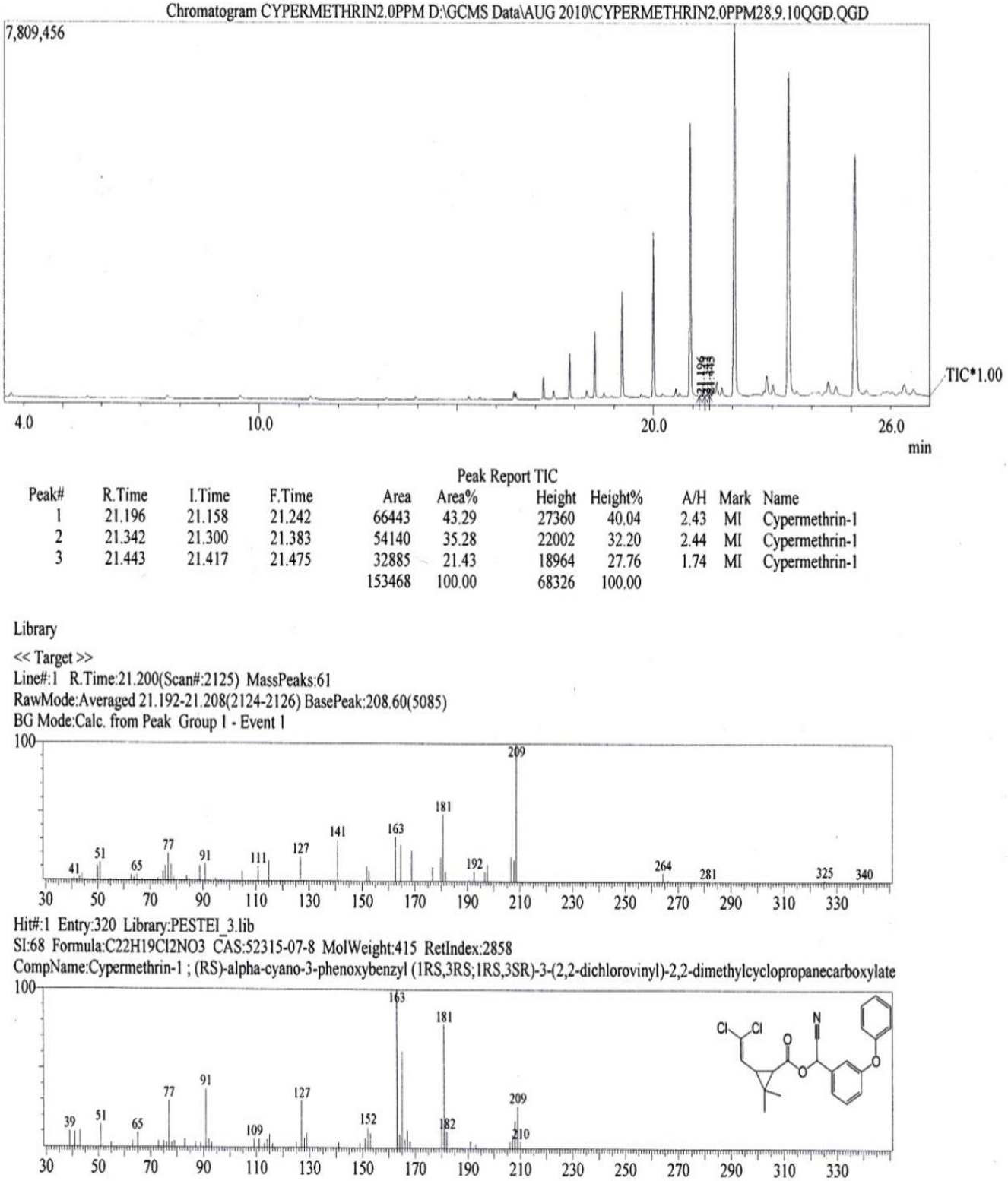

Fig 1: Chromatogram of cypermethrin on GCMS

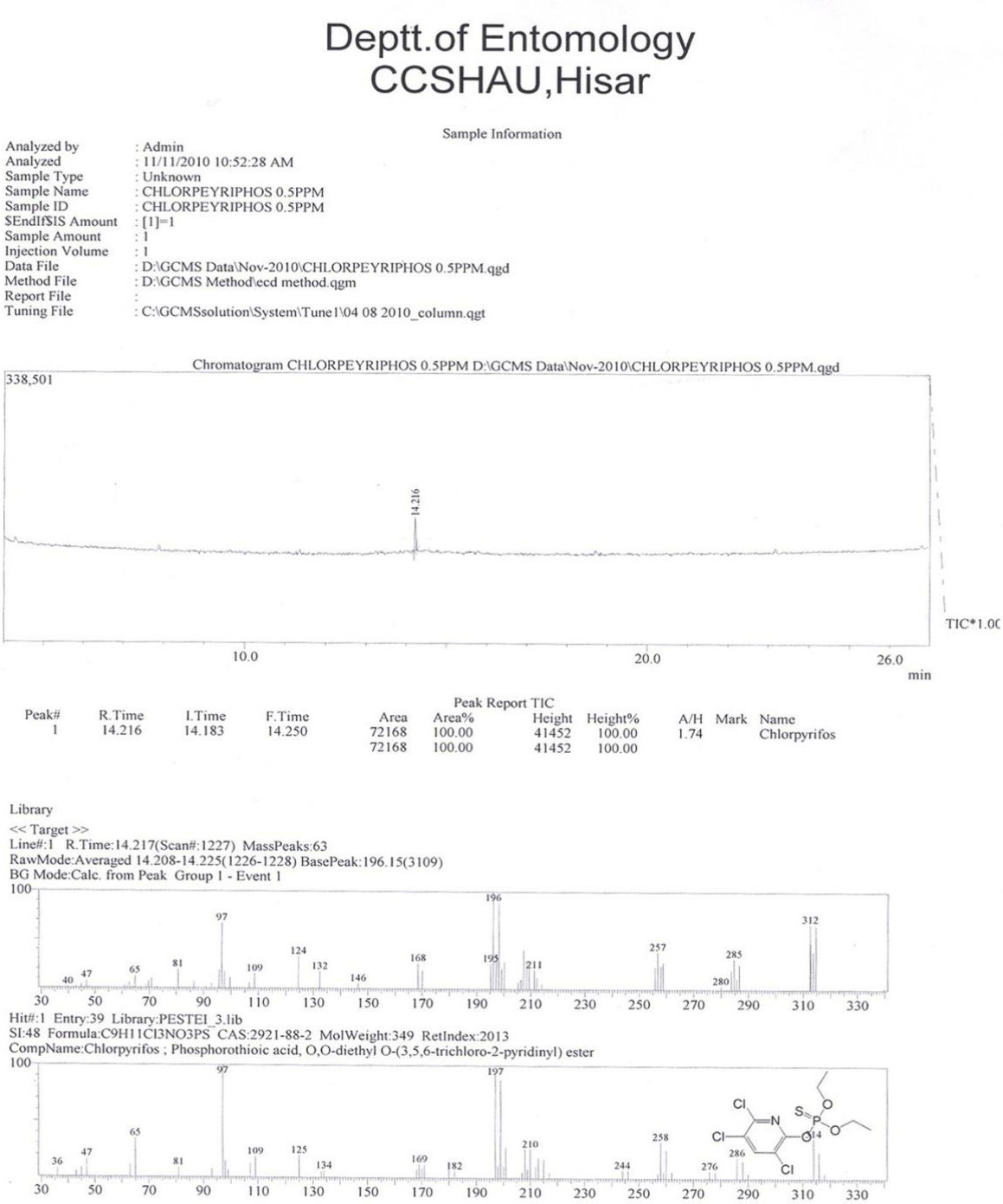

Fig 2: Chromatogram of chlorpyriphos on GCMS

Results and Discussion

Persistence Study

The residue data for okra obtained from two treatments i.e. 275 g a.i. ha^{-1} (T_1) and 550 g a.i.ha^{-1} (T_2) for chlorpyriphos in ready-mix is presented in Table 2 and revealed that initial residues of 0.070 mg $kg^{-1}$from T_1 at zero (1h immediately after treatment) day dissipated progressively, residues further declined to below detection limit (BDL) i.e. 0.010 mg kg^{-1} on 7th day hence depicting 100 percent dissipation. At T_2, starting residues of 0.148 mg kg^{-1}dissipated regularly and on 15th day, the residues completely declined to below detection limit showing 100 percent dissipation. In both the doses, residues of chlorpyriphos in ready-mix were below MRL value of 0.2 mg kg^{-1} on 0 day. The residue half-life values were 2.16 days and 3.27 days for T_1 and T_2 doses, respectively.

Foliar spray of chlorpyriphos was applied on hot pepper at suggested dose and reported that residues dissipated continuously with maximum reduction of 93.70 on 20th day after application with half-life value 4.93 days [21]. The initial foliar deposited concentration of chlorpyrifos ($mgkg^{-1}$) on the six vegetables followed the increasing order of brassica chinensis<lettuce<celery<asparagus lettuce<eggplant <pepper [22]. The results of present research corroborate the findings of authors [23] who reported that the residues of chlorpyriphos dissipated to more than 75% after 10 days at both the dosages with half-life 6.02 days at suggested dose and 5.67 days at double the suggested dose. In tomato and green pea, the residues dissipated below detection limit (0.05 mg kg^{-1}) within 10 days at suggested dose with half life value 2.37 for tomato and 2.83 for green pea [24].

Table 2: Residues (mg kg^{-1})* of chlorpyriphos in ready-mix in okra fruits

Days After Treatment	Suggested dose (T1) (275 g a.i. ha^{-1})		Double of Suggested dose (T2) (550 g a.i. ha^{-1})	
	Average Residues ± SD	% Dissipation	Average Residues ± SD	% Dissipation
0	0.070±0.008	-	0.148±0.008	-
1	0.021±0.005	70.00	0.047±0.005	68.24
3	0.015±0.003	78.57	0.034±0.002	77.02
5	0.011±0.003	84.28	0.025±0.003	83.10
7	< LOQ	100	0.019±0.003	87.16
10	-	-	0.012±0.001	91.89
15	-	-	<LOQ	100
At harvest	-	-	-	-
	Correlation Coefficient r = -0.8795 Regression Equation y=0.6601-0.1395x $t_{1/2}$ = 2.16 days		Correlation Coefficient r = -0.9198 Regression Equationy = 0.9202-0.0920x $t_{1/2}$ = 3.27 days	

CD (P≥0.05) for days = 0.0044, for dose= 0.0022, for days x for dose= 0.006
For regression equation, [Residues (mg kg^{-1}) x 10^3] is taken
* Average ±SD of three replicates; LOQ 0.01 mg/kg, MRL: 0.2 mg/kg

The residue data for okra obtained from two treatments i.e. 275 g a.i. ha^{-1} (T_1) and 550 g a.i.ha^{-1} (T_2) for cypermethrin in ready-mix is given in Table 3 and revealed that initial residues of 0.051 mg kg^{-1} from T_1 at zero (1h just after treatment) day dissipated with time and residues declined to below limit of quantification (LOQ) i.e. 0.01 mg kg^{-1} on 7th day after treatment hence depicting 100 percent dissipation. At T_2, initial residues of 0.110 mg kg^{-1} dissipated completely on 15th day, showing 100 percent dissipation. In both the doses, residues of cypermethrin in ready-mix were below MRL value of 0.2mg kg^{-1} on 0 day. The residue half-life values were 2.74 days and 3.07 days at T_1 and T_2 doses for cypermethrin in ready-mix respectively.

Residues of bifenthrin on okra dissipated completely with percent reduction of 98.63 in suggested dose and 98.95 at double the suggested dose on 15th and 30th day after treatment, respectively, showing half-life 1.58 and 2.18 at respective doses [25]. The dissipation behaviour of cypermethrin in green mustard and found that cypermethrin dissipated quickly, with 29–43% degradation occurring within the first 2 days after the last pesticide application [26]. The initial residues of Bifenthrin dissipated >90 % within the same period in tomato showing half-life period of 1.83 and 2.05 days at respective doses [27].

Statistical Analysis

Statistical analysis of the data revealed that degradation of residues of individual doses of chlorpyriphos and cypermethrin in okra crop is significant with days as residues declined with time. Interaction between duration and doses were also found significant (CD=0.005; p=0.05).This suggested that suggested dose has significantly less residue levels as compared to double the suggested dose and with increase in duration, level of residues also decreases significantly in both the doses resulting in reduced residue levels of cypermethrin in pre-mix after 7 and 15 days of treatment in suggested and double the suggested dose from okra fruits respectively.

Table 3: Residues (mg kg^{-1})* of cypermethrin in ready-mix in okra fruits

Days After Treatment	Suggested dose (275 g a.i. ha^{-1})		Double the Suggested dose (550 g a.i. ha^{-1})	
	Average (Residues ± SD)	% Dissipation	Average (Residues ± SD)	% Dissipation
0	0.051±0.002	-	0.110±0.003	-
1	0.040±0.002	21.56	0.086±0.004	21.81
3	0.021±0.002	58.82	0.047±0.003	57.27
5	0.015±0.005	70.58	0.034±0.004	69.09
7	<LOQ	100	0.019±0.003	82.72
10	-	-	0.012±0.001	89.09
15	-	-	< LOQ	100
At harvest	-	-	-	-
	Correlation Coefficient r = -0.9912 Regression Equation y= 0.6988-0.1097x $t_{1/2}$ = 2.74 Days		Correlation Coefficient r = - 0.9942 Regression Equation y= 1.0133-0.0978x $t_{1/2}$ = 3.07 Days	

CD (P≥0.05) for Days = 0.0033, for dose= 0.0017, for Days x for dose= 0.005
For regression equation, [Residues (mg kg^{-1}) x 10^3] is taken
* Average ±SD of three replicates; LOQ: 0.01 mg/kg, MRL: 0.2 mg/kg

Effect of decontamination

The data presented in Table 4 and 5 revealed that initial deposits of 0.070, 0.021, 0.015 and 0.011 mg $kg^{-1}$for chlorpyriphos at suggested dose (T_1) were reduced to 0.047, 0.023, 0.012 mg kg^{-1} and BDL respectively due to washing of okra fruits resulting in 32.85, 25.80 and 20.00 percent reduction on 0 (1h), 1 and 3 days after treatment respectively and due to washing followed by boiling initial residues further reduced to 0.025 mg kg^{-1} and BDL on 0 (1hr) and 1 day after treatment in okra fruits resulting in 64.28 percent reduction respectively.

In case of washing in double the suggested dose (T_2), the initial residues of 0.148, 0.047, 0.034, 0.025, 0.019 and 0.012 mg kg^{-1} were reduced to 0.096, 0.034, 0.026, 0.020, 0.016 and 0.011 mg kg^{-1} respectively after washing on (1h), 1, 3, 5, 7 and 10 days after treatment and thus showed 35.13, 27.65, 23.52, 20.00, 15.71 and 8.33 percent loss of chlorpyriphos in ready-mix at corresponding time intervals. for washing followed by boiling, the residue reached to the levels of 0.044 and 0.010 mg kg^{-1} after 0 (1h) and 1 days after treatment and

obtained 70.27 and 78.72 percent loss residues at corresponding time intervals.

The decontamination behaviour of chlorpyriphos by washing alone found effective in reducing the residues by 67.75 and 71.60 percent at, respective doses [23]. Household processing was very effective in reducing the levels of chlorpyriphos residues in okra fruits. Maximum reduction (64–77%) was observed by washing followed by boiling then washing (13–35%) alone [28].

Table 4: Effect of processing on reduction of residues of chlorpyriphos in ready-mix insecticide in okra at suggested dose

Days After Treatment	Suggested Dose(550g a.i./ha)		
	Average Residues (mg kg^{-1})*± SD	Washing	Washing + Cooking
0	0.070±0.008	0.047±0.004 (32.85)	0.025±0.003 (64.28)
1	0.031±0.005	0.023±0.003 (25.80)	0.007±0.002 (77.41)
3	0.015±0.003	0.012±0.001 (20.00)	-
5	0.011±0.003	0.009±0.001 (18.18)	-
7	0.008±0.002	-	-
10	0.005±0.002	-	-
15	-	-	-
At harvest	-	-	

* Average ±SD of three replicates;
Figs. in parentheses is percent reduction of residues in comparison to initial deposits

Table 5: Effect of processing on reduction of residues of chlorpyriphos in ready-mix insecticide in okra at double the suggested dose

Days After Treatment	Double the Suggested Dose (1100g a.i./ha)		
	Average Residues (mg kg^{-1})*± SD	Washing	Washing + Cooking
0	0.148±0.001	0.096±0.007 (35.13)	0.044±0.009 (70.27)
1	0.047±0.005	0.034±0.007 (27.65)	0.010±0.002 (78.72)
3	0.034±0.002	0.026±0.005 (23.52)	BDL
5	0.025±0.003	0.020±0.004 (20.00)	-
7	0.019±0.003	0.016±0.003 (15.71)	-
10	0.012±0.001	0.011±0.003 (8.33)	-
15	0.007±0.002	-	-
At harvest	-	-	-

* Average ±SD of three replicates;
Figs. in parentheses is percent reduction of residues in comparison to initial deposits

The data presented in Table 6 and 7 revealed that at suggested dose (T_1) residue levels of 0.051, 0.040, 0.021 and 0.015 mg kg^{-1} as observed on 0 (1h), 1, 3 and 5 days after treatment were reduced to 0.033, 0.030, 0.017 and 0.013 mg kg^{-1} respectively resulting in 35.29, 25.00, 19.04 and 13.33 percent reduction due to washing however when samples were subjected to washing followed by boiling initial residues levels reduced to 0.022 and 0.014 mg kg^{-1} on 0 (1hr) and 1 day after treatment in okra fruits resulting in 56.86 and 65.00 percent reduction respectively. After 3 days residues were below detectable limit. In case of double the suggested dose (T_2), the initial residue of 0.110, 0.086, 0.047, 0.034, 0.019 and 0.012 mg kg^{-1} were reduced to 0.068, 0.062, 0.038, 0.029, 0.017 mg kg^{-1} respectively on (1h), 1, 3, 5, 7 and 10 days after treatment due to washing and thus showed 39.49, 27.77, 20.89, 16.66, 10.52 and 8.33 percent loss at corresponding time intervals. Due to washing followed by boiling residues reached to the levels of 0.042, 0.029, 0.014 mg kg^{-1} and BDL after 0 (1h), 1, 3 and 5 days after treatment obtaining 61.81, 66.27 and 70.21 percent loss of cypermethrin residues in ready-mix at corresponding time intervals.

Present results are in agreement with author [25] who reported that processing was found quite effective in reducing the levels of bifenthrin residues in okra fruits. Maximum reduction (64.58 to 68.42%) was observed by washing + boiling followed by washing (36.71 to 40.00%). Similar results were reported in brinjal [29] in which maximum reduction (31–42%) and (26–37%) was observed by washing followed by boiling/cooking for cypermethrin and decamethrin, respectively. Effects of processing like washing, washing followed by boiling were studied to dislodge the residues of lambda-cyhalothrin on tomato fruit [30] and found that Washing followed by boiling reduced the residues from 72 to 80% whereas only washing reduced the residues from 35 to 36%. Similar results were shown [27].

Table 6: Effect of processing on reduction of residues of cypermethrin in ready-mix insecticide in okra at suggested dose

Days After Treatment	Suggested dose (275g a.i./ha)		
	Average Residues (mg kg^{-1})*± SD	Washing	Washing + Cooking
0	0.051±0.002	0.033±0.004 (35.29)	0.022±0.004 (56.86)
1	0.040±0.002	0.030±0.003 (25.00)	0.014±0.001 (65.00)
3	0.021±0.002	0.017±0.002 (19.04)	
5	0.015±0.005	0.013±0.002 (13.33)	-
7	BDL	-	-
10	-	-	-
15	-	-	-
At harvest	-	-	

* Average ±SD of three replicates;
Figs. in parentheses is percent reduction of residues in comparison to initial deposits

Table 7: Effect of processing on reduction of residues of cypermethrin in ready-mix insecticide in okra at double the suggested dose

Days After Treatment	Double the Suggested Dose (550g a.i./ha)		
	Average Residues (mg kg^{-1})*± SD	Washing	Washing + Cooking
0	0.110±0.003	0.068±0.002 (39.49)	0.042±0.003 (61.81)
1	0.086±0.004	0.062±0.004 (27.77)	0.029±0.003 (66.27)
3	0.047±0.003	0.038±0.002 (20.89)	0.014±0.002 (70.21)
5	0.034±0.004	0.029±0.005 (16.66)	
7	0.019±0.003	0.017±0.003 (10.52)	-
10	0.012±0.001	0.011±0.001 (8.33)	-
15	BDL	-	-
At harvest	-	-	-

* Average ±SD of three replicates;
Figs. in parentheses is percent reduction of residues in comparison to initial deposits

Conclusion

Residues of cypermethrin in okra fruits were below MRL value of 0.2 mg kg^{-1} on zero day in suggested dose and 1 day in double the suggested dose. Additionally decontamination processes indicated that the levels of cypermethrin residues could 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 consumer‘s safety.

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