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Residues, risk assessment and decontamination of lambda cyhalothrin residues from tomato fruits

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Abstract
Lambda cyhalothrin is a synthetic pyrethroid insecticide, recommended in tomato for the control of fruit borer in the form of 5% EC and 4.9% CS @15 g a.i ha^{-1} by CIBRC with the Pre Harvest Interval of 4 and 5 days for respective formulations. Neither FSSAI nor CAC fixed MRL values for lambda cyhalothrin on tomato. In this view a research project was taken to study dissipation pattern of lambda cyhalothrin in both open fields and poly houses, and to address the food safety issue, common household decontamination methods were evaluated for their efficiency in removing lambda cyhalothrin residues from tomato fruits. Residues were estimated through regular sampling till the residues reach below determination level (BDL) of 0.05 mg kg^{-1}, following the validated QuEChERS method. Initial deposits of 0.18 mg kg^{-1} and 0.13 mg kg^{-1} were detected in tomato samples collected from the poly house and open fields which dissipated to BDL by the 7th and 5th day respectively. MRL of 0.3 mg kg^{-1} is recommended by utilizing the OECD MRL calculator, and chronic hazard exposure assessment was carried out based on average body weight, national per capita consumption of tomato and acceptable daily intake (ADI) of lambda cyhalothrin. Among various decontamination methods tested, veggy wash found to be very effective in removing lambda cyhalothrin residues to an extent of 68.87% which can be recommended as risk mitigation method for food safety, followed by 4% acetic acid solution (59.31%) and 2% salt solution (48.02%).

Keywords: Tomato, lambda cyhalothrin, residues, dissipation, MRL

1. Introduction
Lambda cyhalothrin is a synthetic pyrethroid insecticide, effective against tomato fruit borer *Helicoverpa armigera* and tobacco caterpillar *Spodoptera litura* [2, 8]. It is recommended in tomato for the control of fruit borer in the form of 5% EC and 4.9% CS @15 g a.i ha^{-1} by CIBRC with the Pre Harvest Interval of 4 and 5 days for respective formulations [7]. Pesticide residues in fruits and vegetables has affected exports in recent years and should be strictly monitored owing to the high concern about the toxic properties of residues. Maximum Residue Limit (MRL) often serve as safety limit that define the maximum expected level of a pesticide on a food commodity after its safe and authorized use. The limits are food specific and serve as monitoring tools. They serve both to prevent illegal and/or excessive use of a pesticide and act as an enforcement tool to ensure compliance with its registered label[3]. Neither FSSAI nor CAC fixed MRL values for lambda cyhalothrin on tomato. Further, with the intensive use of pesticides in poly house crops, residues may be accumulated at levels higher than those permitted by the regulatory authority. The risk of pesticide residues in foods need to be addressed as per FSSAI (Food Safety and Standards Authority of India) and hence for the protection of consumer health and interests, household risk mitigation methods for removal of pesticide residues in tomato are to be recommended based on the scientific evaluation, as the food habits are changing enormously. Keeping these important issues of concern, the present study was planned to study the dissipation kinetics and risk assessment of lambda cyhalothrin in tomato grown under the poly house and open field conditions and to evaluate decontamination methodologies for removal of lambda cyhalothrin residues.

2. Materials and Method
2.1 Chemicals and Reagents
Certified Reference Materials (CRM) of lambda cyhalothrin (96.9% purity) were procured from M/S Sigma Aldrich, Germany, and primary, intermediary and working standards were

prepared from the CRMs using GC PR grade acetone and hexane as solvents. Working standards of were prepared in the range of 0.01 ppm to 0.5 ppm in 10 mL calibrated graduated volumetric flask using distilled n-hexane as solvent. Primary Secondary Amine (Agilent), magnesium sulfate anhydrous (Emsure grade of Merck), sodium sulfate anhydrous (Emparta ACS grade of Merck), acetonitrile (HPLC gradient grade of Merck), acetic acid glacial (HPLC grade of Merck), acetone (Emplure grade of Merck), n-hexane (HPLC grade of Merck) were used during the study for sample preparation. Lambda cyhalothrin 5% EC was procured from local market.

2.2 Analytical Instruments and Limits of Detection

The working standards of lambda cyhalothrin were in injected Gas Chromatograph with Electron Capture Detector (ECD) and Thermionic Specific Detector (TSD) for estimating the lowest quantity of these pesticides which can be detected with injector split ratio of 1:10 under standard operating parameters (Table 1). It was found that the LOD (limit of detection) for lambda cyhalothrin is 0.01 ng, and the linearity is in the range of 0.01 ng to 0.10 ng.

2.3 Method validation

Prior to field experiments, QuEChERS (Quick Easy Cheap Effective Rugged Safe) method for extraction and clean-up was validated as per SANCO/12571/2013 guidelines. Tomato fruits (5 kg) collected from control plots were homogenized with high volume homogenizer (Robot Coupe Blixer 7L) and 15 g was taken in to 50 mL centrifuge tubes. The required quantity of lambda cyhalothrin intermediary standards is added to each 15 g sample to get fortification levels of 0.05 mg kg^{-1}, 0.25 mg kg^{-1} and 0.5 mg kg^{-1} in three replications each. 30±0.1 mL acetonitrile was added to the tube, and sample was homogenized for 2-3 min using Heidolph silent crusher (low volume homogeniser). Then 3±0.1g sodium chloride was added to tube and mixed by shaking gently, and centrifuged for 3 min at 2500-3000 xg with Remi R-238 to separate the organic layer. The top organic layer of about 16 mL was taken into the 50 mL centrifuge tube to which 9±0.1 g anhydrous sodium sulphate was added to remove the moisture content. 8 mL of extract was taken in to 15 mL tube containing 0.4±0.01g PSA sorbent (for dispersive solid phase d-SPE clean up) and 1.2±0.01 g anhydrous magnesium sulphate, and the sample tube was vortexed for 30 sec followed by centrifugation for 5 min at 2500-3000 xg. The extract of (2mL) was transferred into test tubes and evaporated to dryness using concentration work station (Turbovap LV of Caliper life sciences) with nitrogen gas and reconstituted with 1mL n-Hexane:Acetone (9:1) for lambda cyhalothrin analysis. Tomato samples fortified with lambda cyhalothrin 0.05 mg kg^{-1}, 0.25 mg kg^{-1} and 0.5 mg kg^{-1} were analysed and the mean recovery of the residues using the method was 91.44%, 105.08 and 113.46% respectively and the results show that the method is suitable for the analysis of lambda cyhalothrin residues up to
0.05 mg kg^{1}, and the limit of quantitation (LOQ) is 0.05 mg kg^{-1}. Fortification and recovery test results were presented in Table 2. The residues detected below 0.05 mg kg^{-1} were mentioned as levels Below Determination Level (BDL) in all cases.

2.4 Field experiments and sample collections

Tomato crop (Popular hybrid Nirupama) was raised in both poly house and open field laid out in Randomized Block Design at spacing of 60×45 cm with each plot size of 20 m^2 and all Good Agricultural Practices (GAPs) recommended by University were followed. Lambda cyhalothrin 5% EC procured from local market was sprayed @ 15 g a.i. ha^{-1} twice; first spray at fruit initiation stage followed by second spray at 10 days after first spray, using high volume knapsack sprayer with a spray solution of 500 L ha^{-1}. Pest damage free and crack free tomato fruits of 5 kg were collected from each plot in separate polythene bags and brought to laboratory. Samples were collected at regular intervals i.e. 0, 1, 3, 5, 7, 10, 15, 20 days after last spray for dissipation studies. For evaluation of decontamination methods, zero day samples were collected separately in large quantities and made into 6 sets, each in 4 replications. One set of sample is analyzed for initial deposits of flubendiamide. The remaining sets of samples were subjected to various decontamination methods separately and the residues were calculated to know the efficiency of the various decontamination methods in removal of pesticide residues from the tomato samples. The decontamination methods selected for evaluation of efficiency in removal of lambda cyhalothrin residues from tomato are presented in Table 3. After decontamination treatments, the samples were shade dried for 10 min placing on clean blotting papers and analyzed for residues remaining on tomato.

2.5 Calculation Methods

In order to calculate the rate of degradation and half-life of lambda cyhalothrin on tomato fruits, Hoskin's (1961) [5] linear regression equation was followed.

$Y = a + b X$

Where,

Y - Log of tolerance limit

a - Log of initial deposit

b - Slope of the regression line

OECD (Organization for Economic Cooperation and Development) MRL calculator [10].was used for calculation of MRL and chronic hazard risk analysis was performed using TMDI (Theoretical Maximum Daily Intake) for arriving at MRL for recommendation taking in to consideration of national per capita tomato consumption, average body weight and ADI of lambda cyhalothrin. In case of decontamination studies, per cent removal of lambda cyhalothrin was calculated.

3. Results and Discussion

Tomato fruits collected at regular intervals from lambda cyhalothrin sprayed research plots of open field and poly house was analyzed and the data is presented in Table 4. In the poly house, an initial deposit of 0.18 mg kg^{-1} lambda cyhalothrin was detected at 2 hours after last spray, which dissipated to Below Determination Level (BDL) of 0.05 mg kg^{-1} by the 7^{th} day after last spraying on tomato. The initial deposits were dissipated to 0.10, 0.07 and 0.06 mg kg^{-1} by 1, 3 and 5 days after last spray, respectively. The dissipation pattern showed decrease of residues from first day to 5^{th}day. The residues dissipated by 44.44, 61.11 and 66.66% at 1, 3 and 5 days, respectively. Where as in case of open field studies initial deposit of 0.13 mg kg^{-1} lambda cyhalothrin were detected at 2 hours after last spray, which dissipated to Below Determination Level (BDL) of 0.05 mg kg^{-1} by 5^{h} day after last spraying on tomato in open field trials. The initial deposits were dissipated to 0.08 and 0.06 mg kg^{-1} by 1, and 3 days after last spray, respectively. The dissipation pattern showed the decrease of residues from first day to 3^{rd} day and residues dissipated by 38.46 and 53.84% at 1 and 3 days,

respectively**.** In India, as per Food Safety and Standards Authority of India (FSSAI) MRLs are not fixed for lambda cyhalothrin on tomato. Hence, risk analysis is necessary for setting MRLs based on supervised field trials. Based on the present studies both in the poly house and open fields, as per OECD calculator, MRL of 0.3 mg kg^{-1} can be suggested since the TMDI calculated based on OECD is not more than the ADI of 0.02 mg kg body weight. The suggested MRL of 0.3 mg kg^{-1} is in line with the codex MRL of lambda cyhalothrin on cherries (0.3 mg kg^{-1}) and fruiting vegetables other than cucurbits (0.3 mg kg^{-1}).

The above findings are in agreement with the research reports [6] that lambda cyhalothrin sprayed @ 15 g a.i. ha^{-1} on tomato fruits recorded initial deposit of 0.38 mg kg^{-1} and residues reached Below Determination Level (BDL) by 7^{th} day, they suggested waiting period of 3 days. At double the recommended dose i.e. @ 30 g a.i. ha^{-1}, initial deposit of 0.52 mg kg^{-1} dissipated to BDL by the 10^{th} day, with half-life of 3.7 days. The findings of present investigations are also in line with research reports [9] that lambda cyhalothrin sprayed @ 0.005% on brinjal reported initial deposit of 0.13 mg kg^{-1} and residues reached Below Determination Level (BDL) by the 8^{th} day and suggested waiting period was 1 day.

The dissipation dynamics of lambda cyhalothrin on tomato in both open filed and poly house are in first order kinetics, and are in agreement with the research findings [1] where, on brinjal at both recommended (@ 15 g a.i. ha^{-1}) and double dosage (@ 30 g a.i. ha^{-1}) the initial deposits of 0.75 mg kg^{-1} dissipated to BDL in 10 days, and 1.27 mg kg^{-1} dissipated to BDL in 10 days, respectively. Further, it was also reported that the initial deposits may vary with formulation for the same dosage.

The initial deposits and dissipation vary from crop to crop depending up on the crop canopy, season, age of the crop, sample matrix, the surface area of sample etc., and the same can be witnessed based on the test reports on various crops at different doses[1, 11, 14, 15].

The efficiency of various risk mitigation methods for removal of Lambda cyhalothrin residues from tomato is presented in Table 6. The percentage removal of lambda cyhalothrin residues from tomato when subjected to different decontamination solutions at 2 hours after spraying showed that dipping fruits in veggy wash solution for 10 min followed by tap water wash for 30 sec were found to be more effective (68.87%) than other treatments. Acetic acid solution of 4% (59.31%) was found to be next promising treatment, followed by 2% salt solution (48.02%), 0.1% baking soda solution (39.59%) and tap water wash (29.43%).

The present findings are in agreement with research reports that [13] maximum percent removal (52.50%) of lambda cyhalothrin residues from brinjal fruits achieved by bio wash treatment which contains similar ingredients as veggy wash used in our studies, followed by 2% salt solution (47.90%), 0.1% baking soda solution (30.40%). Furthermore reports reveal that highest percent removal of lambda cyhalothrin residues from tomato fruits was achieved by 2% salt solution (58.50%) followed by veggy wash(56.30%), 0.1% baking soda solution (40.50%) and acetic acid solution of 4% (29.80%) [4]. Maximum percent removal of lambda cyhalothrin from chilli fruits by veggy wash treatment (60.12%), followed by acetic acid solution of 4% (53.60%), 2% salt solution (40.96%), 0.1% baking soda solution (36.13%) and tap water (28.74%) were observed[12] which are in agreement with the findings of present investigation.

Table 1: Standard operating parameters of GC

Gas Chromatograph	**Gas Chromatography- AGILENT- 7890B**
Column	VF-5ms Capillary Column 30 m length, 0.25 mm Internal Diameter, 0.25 µm film thickness; 1% methyl siloxane
Column Oven (^{0}C)	Initial 50 ^{0}C for 1 min - increase @ 20 ^{0}C/min upto 325 ^{0}C – hold for 14 min
Detectors	Electron Capture Detector (ECD) Thermionic Specific Detector (TSD)
Detector Temperature (^{0}C)	300
Injector Temperature (^{0}C)	280
Injector Status	Split Ratio: 1:10
Carrier Gas	Nitrogen, Iolar II, Purity 99.999%
Carrier Gas Flow (ml min^{-1})	1 ml min^{-1}
Make-up Flow (ml min^{-1})	35 ml min^{-1}
Retention time (min)	Lambda cyhalothrin: 6.99 min
Total run time (min)	28.75 min

Table 2: Recovery of lambda cyhalothrin residues from tomato

Replications	**Fortified level (**mg kg^{-1}**)**					
	0.05 mg kg^{-1}		**0.25 mg kg^{-1}**		**0.50 mg kg^{-1}**	
	Residues recovered (mg kg^{-1})	**Recovery%**	**Residues recovered (mg kg^{-1})**	**Recovery%**	**Residues recovered (mg kg^{-1})**	**Recovery%**
R1	0.047	94.66	0.267	106.74	0.549	109.87
R2	0.046	91.57	0.256	102.47	0.594	118.71
R3	0.044	88.10	0.265	106.03	0.559	111.79
Mean		91.44		105.08		113.46
SD		3.284		2.291		4.648
RSD		3.592		2.181		4.096

Table 3: Decontamination Methods for removal of lambda cyhalothrin residues from tomato

S. No	Treatment	Details of treatment
T_1	Tap water wash	4 L of tap water was taken into the plastic tub of 7 L capacity and 2 Kg of tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.
T_2	Soaking in 2% salt solution	4 L of 2% salt solution was prepared by mixing 80 g of table salt in 4 L of water in plastic tub of 7 L capacity and 2 Kg tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.
T_3	Dipping in 0.1% baking soda	4 L of 0.1% baking soda solution was prepared by mixing 4 g of baking soda in 4 L of water in plastic tub of 7 L capacity and 2 Kg tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.
T_4	Soaking in 4% acetic acid	4 L of 4% acetic acid solution was prepared by mixing 160 ml of acetic acid glacial 100% in 4 L of water in plastic tub of 7 L capacity, mixture was kept for 1 min and 2 Kg of tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.
T_5	Veggy wash	4 L of veggy wash was prepared by mixing 160 ml of acetic acid glacial 100%, 4 g of baking soda and lemon juice of 4 lemons in 4 L of water in plastic tub of 7 L capacity, mixture was kept for 1 min and 2 Kg tomato fruits were dipped in the tub for 10 min, followed by the tap water wash for 10 sec.

Table 4: Dissipation of lambda cyhalothrin residues in poly house and open field situations

Replication	Residues in Poly House (mg kg^{-1})						Residues in Open field(mg kg^{-1})					
	R1	R2	R3	R4	Mean	% dissipation	R1	R2	R3	R4	Mean	% dissipation
0	0.18	0.18	0.18	0.17	0.18	0	0.13	0.13	0.13	0.13	0.13	0
1	0.11	0.10	0.10	0.10	0.10	44.44	0.09	0.09	0.08	0.08	0.08	38.46
3	0.07	0.07	0.07	0.07	0.07	61.11	0.06	0.05	0.05	0.06	0.06	53.84
5	0.05	0.06	0.06	0.05	0.06	66.66	BDL	BDL	BDL	BDL	BDL	100.00
7	BDL	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	BDL	100.00
10	BDL	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	BDL	100.00
15	BDL	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	BDL	100.00
20	BDL	BDL	BDL	BDL	BDL	100.00	BDL	BDL	BDL	BDL	BDL	100.00
Regression equation	Y = 0.148 + (-0.021) X						Y = 0.121 + (-0.024) X					
R^2	0.867						0.957					
(BDL) Below Determination Level : < 0.05 mg kg^{-1}												

Table 5: Chronic hazard exposure assessment for recommending lambda cyhalothrin MRLs on tomato

OECD MRL calculator Data sets	Poly house	Open field	OECD MRL calculator Data sets	Poly house	Open field	Risk Analysis	
Total number of data (n)	5	4	**Proposed MRL estimate**			Daily intake of crop (C) = kg person $^{-1}$	0.027
Percentage of censored data (%)	20	25	Highest residue	0.180	0.130	Consumption of crop C($F_{C)}$ = kg bw^{-1}	0.00049
Number of non-censored data	4	3	Mean + 4 SD	0.303	0.222	ADI for lambda cyhalothrin (mg kg bw^{-1})	0.02
Lowest residue	0.050	0.050	CF X 3 Mean	0.239	0.200	TMDI = Fc X MRL (from OECD calculator)	0.000147
Highest residue	0.180	0.130	Unrounded MRL	0.303	0.222	TMDI v/s ADI	TMDI < ADI
Median residue	0.070	0.070	Rounded MRL	0.3	0.3	Proposed MRL (mg kg^{-1})	0.3
Mean	0.092	0.080	**Risk Analysis**			Codex MRL (mg kg^{-1})	Not available
Standard deviation (SD)	0.053	0.036	Average human body weight (kg)	55		FSSAI (India) MRL (mg kg^{-1})	Not available
Correction factor for censoring (CF)	0.867	0.833	National per capita intake of tomato	806 g month^{-1}		EU MRL (mg kg^{-1})	0.1

Table 6: Removal of Lambda cyhalothrin residues from tomato fruits with different decontamination methods

Treatments	Mean of Lambda cyhalothrin detected (mg kg-1)*	Amount removed (mg kg-1) **	Percent removed
Tap water wash	0.09 ± 0.004	0.054 ± 0.030	29.43 ± 2.81
2% salt solution	0.07 ± 0.003	0.064 ± 0.010	48.02 ± 2.14
0.1% Baking soda solution	0.08 ± 0.003	0.063 ± 0.017	39.59 ± 2.50
4% Acetic acid solution	0.05 ± 0.004	0.069 ± 0.014	59.31 ± 0.05
Veggy wash	0.04 ± 0.004	0.072 ± 0.029	68.87 ± 2.19
C. D. at 5% = 3.89; Initial deposit = 0.13 mg kg^{-1}; * Mean of three replications; ** Amount removed = Initial deposit-Mean of replicates of each treatments.			

4. Conclusions

Dissipation pattern of lambda cyhalothrin varies from open field to poly house conditions when sprayed as per farmers practice. Lambda cyhalothrin is recommended for use on tomato against fruit borers as per Insecticide Act, 1968 @ 15 g a.i. ha^{-1} and Food Safety and Standards Authority of India (FSSAI) and Codex Alimentarius Commission doesn't fixed MRL for lambda cyhalothrin on tomato. Based on the present studies both in poly house and open fields, as per OECD calculator, MRL of 0.3 mg kg^{-1} can be suggested since the

TMDI calculated based on OECD is not more than the ADI of 0.02 mg kg body weight. The suggested MRL of 0.3 mg kg^{-1} is in line with the codex MRL of lambda cyhalothrin on cherries (0.3 mg kg^{-1}) and fruiting vegetables other than cucurbits (0.3 mg kg^{-1}). Further decontamination methodologies varies in their efficiency of removal of residues from treated surfaces across commodities, but in general veggy wash, 2% salt solution, and 4% acetic acid/vinegar solution, is suggested for use, which can be prepared by house hold materials.

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