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Dissipation of Lambda-cyhalothrin 5% EC on Brinjal Fruit Grown in Gurugram, Haryana

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ABSTRACT

Background: The introduction of synthetic pesticides Lambda-cyhalothrinin for crop protection in general has significantly contributed to increased productivity. Lambda-cyhalothrin have been reported to cause toxic effects on humans, ranging from short-term effects such as headaches and nausea to chronic effects like cancer, reproductive damage and endocrine disruption. Lambda-cyhalothrin is a type-II highly active synthetic pyrethroid insecticide which contains a-cyano group. Lambda-cyhalothrin applied at the rate of 30 g *a.i.* ha^{-1} was found to be effective against shoot and fruit borer on brinjal.

Methods: The analytical method for the determination of Lambda-cyhalothrin 5% EC (Test Item) on brinjal was validated as per DG-SANTE 2019 guideline. The field trails including the dissipation study and final residue study were conducted in a randomized block design in open field. As per recommendation, dose of test item (T_1=15 g *a.i*/ha; T_2=30g a.i/ha) were 500 L/hectare for Trial. Lambda-cyhalothrin residues of brinjal samples were determined at 0, 1, 3, 5, 7 and 10 days at both application of recommended and double the recommended dose of test item.

Result: The analytical method employed for determination of persistence residues of Lambda-cyhalothrin in brinjal was validated and found accurate and precise. The per cent dissipation of Lambda-cyhalothrin for the treatment T_1 was 37.22% after 1 day which increased to 96.66% by 10^{th} day, similarly for the double dose (T_2) the initial dissipation was 57.14%, which increased to 99.7% after 10^{th} day. The half-life ($T_{1/2}$) values of Lambda-cyhalothrin on brinjal were found to be 2.06 and 2.13 days for T_1 and T_2, respectively.

Key words: Brinjal, Lambda-cyhalothrin, Per cent dissipation, Persistence residues.

INTRODUCTION

Lambda-cyhalothrin is (R)-α-cyano-3-phenoxybenzyl (1S)-cis-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethyl cyclopropane carboxylate and (S)-a-cyano-3-phenoxy benzyl(1R)-cis-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2 dimethyl cyclopropane carboxylate. It belongs to a group of chemicals called pyrethroids. Pyrethroids are manmade chemicals that are similar to the natural insecticides pyrethrins. The introduction of synthetic pesticides in crop protection in general has significantly contributed to increased productivity (Pretty, and Bharucha, 2015). After application, pesticides tend to degrade, with their residues remaining not only in the plants but also in various environmental matrices, like water, soil or sediments. Pesticides have been reported to cause toxic effects on humans, ranging from short-term effects such as headaches and nausea to chronic effects like cancer, reproductive damage and endocrine disruption (Berrada *et al.,* 2010; Claeys *et al.,* 2011; Malhat *et al*., 2014a).

The maximum residue limit (MRL) of pesticides for each commodity is determined by the Codex Alimentarius Commission (CAC) and FSSAI (Food Safety and Standards Authority of India). The MRL serves as one of the quality control parameter for protecting the health of consumers of agricultural products while facilitating international trade (FAO/WHO 2003). Maximum residue levels (MRLs) are useful parameters that promote food safety by restricting the concentration of a pesticide residue permitted on a commodity (Chen *et al.,* 2011; Claeys *et al.,* 2011; Malhat and Hassan, 2011). MRL standards are used worldwide to promote safer food and maintain Acceptable Daily Intake of pesticides. Information about the dissipation rate of a pesticide after application is a valuable tool for assessing the behaviour of residues and estimating the preharvest interval (PHI) (Malhat *et al*., 2014a). Dissipation kinetics and residue analysis of Lambda-cyhalothrin in green beans and spring onion (Hanafi *et al.,* 2010), tomato (Jayakrishnan *et al.,* 2005) and grapes (Banerjee *et al.,* 2006) have been studied in details against various insect-pests.

The present study was carried out to access the dissipation of Lambda-cyhalothrin on the field and also to develop and validate an analytical method for the detection and quantification of lambda-cyhalothrin, a synthetic pyrethroid insecticide, in brinjal using gas chromatography tandem mass spectrometry (GC-MS/MS). The study has meant to ascertain Lambda-cyhalothrin dissipation behaviour under field conditions.

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MATERIALS AND METHODS

Chemicals

Certified reference standard of Lambda-cyhalothrin (98.80% purity) were purchased from Sigma-Aldrich, USA which were used for the identification and quantification of residues. All solvent used were of residue and HPLC grade and were obtained from Merck (India). Primary secondary amine (PSA, 40 µm, Agilent, USA) and C18 (ODS; Agilent, USA). Anhydrous sodium sulphate (Na_2SO_4) and anhydrous magnesium sulphate ($MgSO_4$) were purchased from Merck (Germany).

Preparation of Lambda-cyhalothrin standard stock solution

- 1.05 mg of Lambda-cyhalothrin (Purity= 98.8%) was weighed and taken in 10 mL volumetric flask.
- Few drops of Acetone were added in order to dissolve the content present in the volumetric flask.
- Volume was made upto the mark with n-hexane.
- A stock solution of the concentration 104 ppm (mg/kg) was prepared.
  Calculation of the standard stock with help of the formula below:
- Potency correction (Actual weight)= Weighted standard/ 100*Purity%= 1.04.
- Hence concentration of Lambda-cyhalothrin=Actual weight/ Volume of V.F×1000= 1.04/10 ml×1000= 104 ppm.

Preparation of Lambda-cyhalothrin working standard

The stock solution (Lambda-cyhalothrin) which was prepared was diluted for Linearity check in the concentration of 10, 50, 100, 150, 250, 500 µg/L for using hexane as diluents by formula:

$$N1V1=N2V2$$

Where,
N1= Normality of solution to be diluted.
V1= Volume of solution taken for dilution.
N2= Normality of solution to be prepared.
V2= Volumetric flask in which standard solution to be prepared.

Preparation of Lambda-cyhalothrin working solutions for recovery experiment

Working solution for recovery experiment was prepared at 5 ppm from stock solution using hexane as diluent for fortification of matrices at LOQ, 5XLOQ and 10XLOQ (*i.e* 50, 250 and 500 µg/L) levels respectively.

Method validation

The analytical method for the determination of Lambda-cyhalothrin 5% EC brinjal was validated as per DG-SANTE 2019 guideline. The parameters *viz*., specificity, linearity, method precision and accuracy (% recovery) was checked before performing the analysis of the samples received from field trial. The analytical method was validated by evaluating quality parameters such as linearity, precision (repeatability), selectivity, limits of detection and quantification and recovery studies values. The linearity (r2) and sensitivity of the method was checked by plotting calibration curve (10, 50, 100, 150, 250 µg/L). Limits of detection (LOD) and quantification (LOQ) were calculated as three and ten times the signal-to-noise ratio, respectively (DG-SANTE 2019). Trueness and precision of the method has also been tested through recovery experiments which was carried out by spiking the respective control matrix at concentration level of 50, 250, 500 µg/L. Precision was evaluated by calculating relative standard deviation. A stock standard solution about (100 mg/L) of Lambda-cyhalothrin was prepared in n-hexane by weighing approximately 1 mg of certified reference material in 10 mL volumetric flask. All standard solutions were stored in the dark at 4°C. Repeatability (expressed as relative standard deviation, RSD%) was determined by analysing samples spiked with Lambda-cyhalothrin at all the concentrations used to determine the linear range on the same day. Triplicate Brinjal samples were spiked with 50, 250 and 500 µg/L of standard solutions of Lambda-cyhalothrin.

Field experiment design

The field trails including the dissipation study and final residue study were conducted in a randomized block design in open field at Institute of Pesticide Formulation Technology (IPFT), Gurugram, Haryana, India. Each experiment was conducted in four separate plots measuring 20 sq.mt each where three plots were used for insecticide treatment and the other plot was used as control (blank sample). None of the plots had been treated with lambda-cyhalothrin in the past. There was no rainfall at any time during the experimental period. The field was prepared for plantation of brinjal plant. The brinjal plants were planted from nursery to field when the plant height was about to 15 cm. The details of the field trial are given in Table 1.

As per recommendation, dose of test item (T_1=15 g a.i/ha; T_2=30 g a.i/ha) were 500 L/hectare for trial. Hence for plot size 5×4 meter square (20 m^2) dose of T_1=0.6 mL×3 plots (Single dose)= 1.8 mL of 5% EC and plot size 5×4 meter square (20 m^2) dose of T_2=1.8×3 plots (double dose) =3.6 mL. Here three plots was prepared for single dose treatment and 3 for double dose treatment and three for untreated control. Hence total 5.4 mL of Lambda-cyhalothrin 5% EC was applied for treatment.

Sample collection

The samples of field trail were collected at 0, 1, 3, 5, 7 and 10th day of spray and kept in refrigerator; Approx. 250 g of brinjal samples were collected in triplicate for each day for single, double dose and untreated sample.

Meteorological data

The meteorological information from locations such as maximum and minimum temperature, maximum and minimum relative humidity, total rainfall and sunshine in the locality of field trials was collected from respective scientists. The study was conducted during month of August-Septembr-2022. The details are given in Table 2.

Analytical parameters

Experimental condition for GC-MS/MS: Pesticide residues of Lambda-cyhalothrin were confirmed by using Gas chromatography mass spectrometry (GCMS/MS) fitted with

Table 1: Field trail details of study carried on brinjal fruit.

Field trial details	
Location of trial	Institute of pesticide formulation technology, Gurgaon-Haryana for bio-efficacy evaluation
Name of the formulation	Lambda-cyhalothrin
Crop	Brinjal
Variety	F1 hybrid madve
Plot size	4 m × 5 m (20 sq. mt)
Plot layout	Randomized block design (RBD)
Date of crop transplanting	13/MAY/2021
Type of application	High volume spray with Knapsack sprayer fitted with hollow cone nozzle and using spray volume @ 500 lit /ha
Date of application	25/AUG/2021
Date of final collection	04/SEP/2021
Sampling time	Brinjal fruits were collected at 0, 1, 3, 5, 7 and 10 days after spray

Table 2: Meteorological data during the experimental period (August/September 2021).

Time	Temperature (°F)			Dew point (°F)			Humidity (%)			Precipitation (in cm)
August	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min	Total
25 Aug	88	72.5	59	61	55.7	50	94	60.5	29	0.00
26 Aug	88	74.2	0	63	55.8	0	94	54.6	0	0.00
27 Aug	86	75.3	66	63	52.9	48	73	47.6	27	0.00
28 Aug	86	76.1	66	54	49.8	43	64	42.0	22	0.00
29 Aug	87	74.2	0	63	55.8	0	94	54.6	0	0.00
30 Aug	86	75.3	66	62	52.9	48	73	47.6	27	0.00
31 Aug	88	76.1	66	54	49.8	43	64	42.0	22	0.00
1 Sep	81	77.8	73	79	77.5	73	100	99.1	94	0.00
2 Sep	90	82.4	75	84	80.0	73	100	93.3	79	0.00
3 Sep	93	84.3	79	82	79.9	77	100	87.1	67	0.00
4 Sep	90	82.8	79	82	80.1	79	100	90.9	79	0.00

DB-5 MS (length: 30 m, I.D: 0.25 mm, film thickness: 0.25 µm) capillary column. Injector temperature was set at 250°C. The column oven temperature was programming set at 50°C held for 2 min and gradually increased with rate 15°C/min to 190°C and held for 1 min then raised at 5°C/min to 280°C for 10 min. MS system includes positive electron ionization (EI) energy mode at 70 eV energy with multiple reaction monitoring (MRM) scan mode. The ion source, interface and quad temperature was maintained at 230, 280 and 150°C, respectively. Helium was used as carrier gas flow at 1 mL/min and 1 µL injection volume was injected at splitless mode. Pesticides in samples were confirmed according to their specific retention time and transition ions (primary and secondary) with assistance of the inbuilt NIST (National Institute of Standards and Technology; Maryland, US) pesticide library.

Extraction and clean up procedure of brinjal sample

About 250 g brinjal samples were homogenized using mixer grinder. About 10 g of homogenized samples were taken using an analytical balance in a 50 ml Teflon centrifuge tube with three replicates of each. 10 ml of acetonitrile was added to each centrifuge tube and vortex for 2 minutes and kept aside for half an hour. 4 g of anhydrous magnesium sulphate ($MgSO_4$) was added to each tube and mixed by manual shaking followed by roto-spin. After half an hour the samples were centrifuged at 3000 rpm for 3 minutes. For clean-up, 5 ml of supernatant solution was transferred into another 15 ml centrifuge tube containing dispersive solid phase extraction (d-SPE) sorbents: 125 mg of primary secondary amine (PSA), 125 mg of C-18 and 750 mg of anhydrous magnesium sulphate ($MgSO_4$) and subjected to vortex for 2 minutes and then centrifuged at 3000 rpm for 3 minutes. After centrifugation, 1 ml upper solvent layer was transferred to 5 ml glass turbo tube and evaporated near to dryness under mild nitrogen stream. Final volume was reconstituted with 1 ml acetonitrile so as to keep the concentration in linear range of response which is filtered the content using 0.22 µm nylon syringe filter in GC-MS/MS vials for injections.

Calculation for method validation samples

Residue concentration in sample (mg/kg) was calculated using the following formula:

$$\text{Residue concentration } (\mu g/g) = C_{sample}\ (\mu g/L) \times d/1$$

C_{sample} = Concentration of sample extract (µg/L) obtained by the instrument.

d = Dilution factor

Recovery % of the residues was calculated using the following formula:

Recovery %= Concentration recovered (µg/L) × 100

Persistence

Day wise dissipation was calculated by using the formula:

$$\text{Dissipation per cent} = \frac{\text{Initial conc at zero day- Conc at specific day}}{\text{Initial conc at zero day}}$$

The regression coefficient (R^2) represents the relationship between residual data with time and residual half life was calculated using formula:

$$t_{1/2} = \ln 2/k.$$

The test results (Result= Instrument reported concentration × dilution factor or divided by concentration factor) regarding the residue of in brinjal are summarized by the regression equation and half-life values for the different treatments. Analysis of the samples was done by injecting calibrating standards of different concentration levels with samples in the same sequence. The sequence was processed and concentrations of samples were calculated.

Residue in brinjal samples

The Lambda-cyhalothrin residues of brinjal samples were determined at 0, 1, 3, 5, 7 and 10 days at both application of recommended and double the recommended dose of Lambda-cyhalothrin 5% EC. No residue levels were detected in control (T3) sample of brinjal sample. The results are presented in Table 3 and representative graphs were given in Fig 1.

RESULTS AND DISCUSSION

Specificity

The method was found specific for quantification of Lambda-cyhalothrin 5% EC on brinjal, as no interference was found w.r.t. LOQ level calibration standard. There were no interferences found when solvent blank, reagent blank and standard 250 ppb were analysed with LOQ level calibration standard. The results of specificity are presented in Table 5.

Linearity

Linearity of the instrument was checked forLambda-cyhalothrin and it was found linear in the range of 10 µg/L-250 µg/L concentrations with R^2 value>0.99. Calibration levels were injected thrice to check the repeatability of injections. The repeatability at each level complies with the guidelines. Retention time accuracy and sensitivity were recorded.

The repeatability at each level complies with the guidelines. Retention time accuracy and sensitivity were recorded. Five calibration solutions of Lambda-cyhalothrin at different concentrations, ranging from 10 µg/L to 500 µg/L were prepared and analyzed in GC-MS/MS. Then linearity was established by plotting response of analyte with concentration (µg/L) (Fig 2). The coefficient of correlation was found more than 0.99.

Limits of detection (LOD) and quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of the method were optimised at 0.01 and 0.05 µg/g, respectively which compiles European Union (EU) protocols (DG-SANTE, 2019). According to the document SANCO/12495/2013 (SANCO, 2013) the limit of quantification should be ≤MRL. hows the maximum residue level (MRL) for Lambda-cyhalothrin 0.2 mg/kg for brinjal (FSSAI, 2019).

Accuracy (% Recovery) and method precision

Recovery studies were carried out in order to establish the authenticity of the analytical method employed. According to EU the recovery study should be in the range of 70-120% with RSD below 20% (DG-SANTE 2019). Lambda-cyhalothrin Brinjal samples were fortified at the level of 50 (LOQ), 250 (5LOQ) and 500 µg/L (10LOQ) with the standard solutions of Lambda-cyhalothrin and were analyzed following the aforementioned procedure. Results of recovery study are shown in Table 6. According to the document SANCO/12571/2013 (SANCO 2013), the acceptable drift between two bracketing injections of the same concentration should not exceed 30%. The three level of 50, 250 and 500 µg/L were injected in triplicates, the repeatable area does not exceeds above 20%. According to document SANCO/12571/2013 (SANCO 2013), acceptable mean recoveries lie within the range of 70-120. The recovered amount of the spiked samples at all the LOQ values lies between 88-91% with relative standard deviation lies below 10%. All the results showing the method are accurate and precise. Detail result of accuracy and precision is given in Table 6.

Recovery was calculated using the following equation:

$$\text{Recovery \%} = \frac{\text{Measured concentration}}{\text{Spiked (added) concentration}} \times 100$$

The precision of the method was determined by assessing repeatability and reproducibility, expressed as RSD% values. The repeatability relative standard deviation (RSDr) was measured by comparing the standard deviation values of the recoveries from spiked samples analyzed on the same day.

$$\text{RSD\%} = \frac{\text{Standard deviation of replicates}}{\text{Mean value of recovery}} \times 100$$

All the results showing the method is accurate and precise.
MMREC-BJ= Matrix match recovery brinjal.
SD= Standard deviation.
RSD= Relative standard deviation.

Residue in brinjal samples

Lambda-cyhalothrin residues of brinjal samples were determined at 0, 1, 3, 5, 7 and 10 days at both application of recommended and double the recommended dose of Lambda-cyhalothrin 5% EC. No residue levels were detected in control (T3) sample of brinjal sample.

Persistence of Lambda-cyhalothrin 5% EC and their half-life of dissipation

For better understanding of the possible hazardous impacts of pesticides residues, dissipation studies are necessary to examine the appropriateness of pesticides application strategies (Lu *et al.,* 2014). Day wise residue data was subjected to first order kinetics by using the formula:

$$C_t = C_0 e^{-kt}$$

Where,

C_t = Denotes the concentration (µg/g) after time t (day).

C_0 = Initial conc.

K = Rate constant (day-1).

The regression coefficient (R^2) represents the relationship between residual data with time and residual half-life was calculated using formula:

$$t_{1/2} = \ln 2/k$$

The test results (Result= Instrument reported concentration × dilution factor or divided by concentration factor) regarding the residue of Lambda-cyhalothrin in brinjal are summarized by the regression equation and half-life values for the different treatments. Analysis of the samples was done by injecting calibrating standards of different concentration levels with samples in the same sequence. The sequence was processed and concentrations of

Table 3: Days wise residues of Lambda-cyhalothrinin brinjal.

Treatment	Days	R1	R2	R3	Residues in ppm (µg/g)		
					Mean	Standard deviation	Per cent dissipation
T_1	0	1.82	1.78	1.8	1.8	0.020	NA
	1	1.08	1.15	1.17	1.13	0.047	37.22
	3	0.66	0.7	0.68	0.68	0.020	62.22
	5	0.38	0.41	0.41	0.4	0.017	77.7
	7	0.13	0.13	0.13	0.13	0.000	92.77
	10	0.062	0.069	0.068	0.066	0.004	96.66
	0	4.25	4.22	4.36	4.28	0.074	NA
	1	1.91	1.82	1.77	1.83	0.071	57.14
	3	1.25	1.29	1.28	1.27	0.021	70.37
	5	0.77	0.81	0.82	0.8	0.026	81.3
T_2	7	0.3	0.3	0.29	0.3	0.006	92.99
	10	0.15	0.13	0.13	0.14	0.012	99.7
	0	BDL	BDL	BDL	-		-
T_3	1	BDL	BDL	BDL	-		-
	3	BDL	BDL	BDL	-		-
	5	BDL	BDL	BDL	-		-
	7	BDL	BDL	BDL	-		-
	10	BDL	BDL	BDL	-		-

Table 4: Regression equation and half-life values of Lambda-cyhalothrinin brinjal.

Location	Treatment	Regression equation	Half-life (Days)
IPFT, Gurugram	T_1	y= -0.1455x+3.2431	2.06
	T_2	y= -0.1408x+3.531	2.13

Table 5: Specificity results for analysis of Lambda-cyhalothrin in brinjal.

Analyte: Lambda-cyhalothrin		
Standard 5LOQ Level Sample		
Sample ID	Analyte retention time (Min) (0.9) RT	Peak area of analyte (A)
Reagent blank	BDL	0
Blank	BDL	0
Control samples		
Sample ID	Analyte retention time (Min)	Peak area of analyte (B)%	Interference w.r.t LOQ B*100/A
Control BJ	26.3	0	0
MMSTD 0.250 PPM	26.3	148842	0

Table 6: Results of accuracy (% recovery) and precision for analyte Lambda-cyhalothrin in brinjal.

Level	Sample ID	Sample wt.(w) in gm	Fortified conc. (X) (µg/kg)	Sample dilution factor (d):(mL/g)	Csample analyte (y) µg/L=X	Recovered conc. (µg/kg) (loq area/ std loq* std conc.)=x	% Recovery x/X*100
1XLOQ	MMREC-BJ1-LOQ1	10.04	50	10 ml	50	47.62	95.25
	MMREC-BJ1-LOQ2	10.03	50	10 ml	50	48.089	96.17
	MMREC-BJ1-LOQ3	10.05	50	10 ml	50	43.50	87.017
	Mean						92.818
	SD						5.039
	RSD						5.430
5XLOQ	MMREC-BJ5-LOQ1	10.02	250	10 ml	250	228.84	91.53
	MMREC-BJ5-LOQ1	10.03	250	10 ml	250	273.82	109.52
	MMREC-BJ5-LOQ1	10.01	250	10 ml	250	262.88	105.15
	Mean						102.070
	SD						9.386
	RSD						9.197
10XLOQ	MMREC-BJ10-LOQ1	10.04	500	10 ml	500	533.70	106.74
	MMREC-BJ10-LOQ1	10.03	500	10 ml	500	472.81	94.56
	MMREC-BJ10-LOQ1	10.05	500	10 ml	500	461.77	92.35
	Mean						97.883
	SD						7.7493
	RSD						7.917

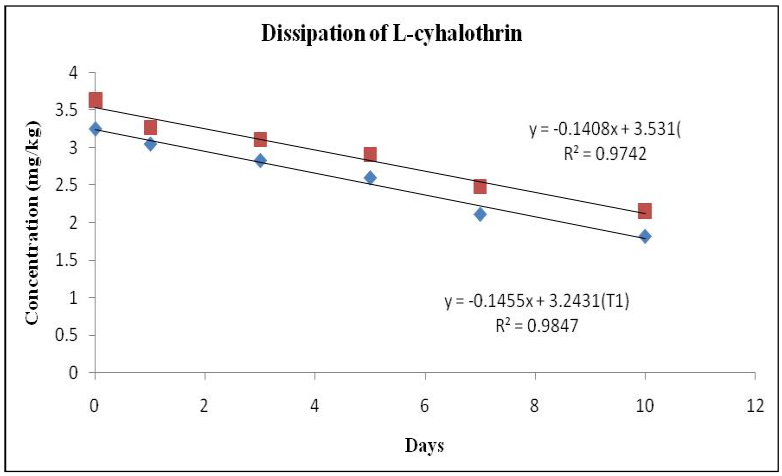

Fig 1: Dissipation pattern of Lambda-cyhalothrin 25% EC on brinjal fruit.

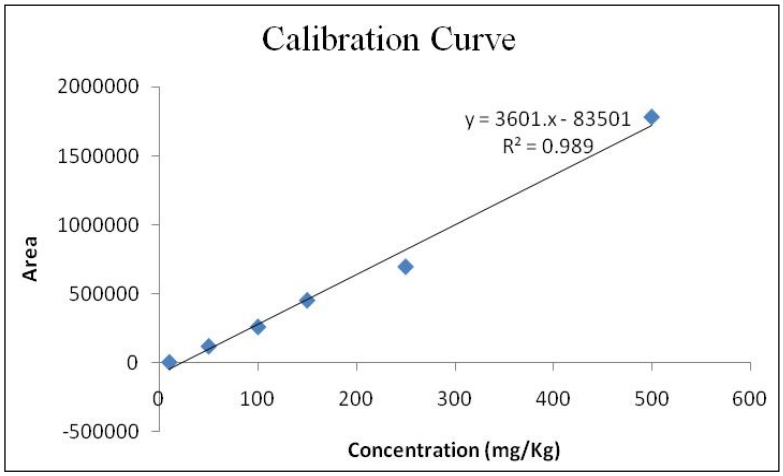

Fig 2: Linearity curve of Lambda-cyhalothrin at 10, 50, 100, 150, 200, 250 and 500 µg/L.

samples were calculated. The persistence residue concentrations are tabulated in Table 3, 4 and Fig 1. The degradation kinetics of Lambda-cyhalothrin in brinjal was determined by plotting residue concentrations against time and the maximum squares of correlation coefficients obtained were used to determine the equation of best-fit curves. The dissipation curves of lambda-cyhalothrin in brinjal under field conditions are shown in Fig 1. The decline of residues was significantly ascribed to the growth diluting effect and the degradation of the pesticide itself. There are many factors that influence the fate of pesticide in plants, including the characteristics of the pesticide (including its over-all stability either as parent compound or metabolites, its volatility, solubility, formulation) and the method and site of application (Aydinalp and Porca, 2004; Voutsas *et al.,* 2005; Brady *et al.,* 2006; Isenring and Madeley, 2006; Cabras *et al.,* 1989; Malhat *et al.,* 2014).

CONCLUSION

The analytical method employed for determination of persistence residues of Lambda-cyhalothrinin brinjal was validated and found accurate and precise. The level of Lambda-cyhalothrin in residue in brinjal were below MRL on 10th Day of spray at both recommended and double the recommended dose levels. Lambda-cyhalothrin residue was found below quantifiable limit in all the control samples. Lambda-cyhalothrin may not create any residual toxicity problem in brinjal. Initial concentration of Lambda-cyhalothrin at 5th days in brinjal was below MRL value according to Codex Alimentarious Commission (2009) which is 3 mg/kg of Indian recommendation irrespective of any dose. So, it may be stated that brinjal may safely be consumed without any hazard.

Conflict of interest: None.

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