



Dissipation and Persistence of Chlorpyrifos 20EC in / on Curry Leaf

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Dissipation of chlorpyrifos 20 EC was studied in/on curry leaf (*Murraya koenigii*). Two rounds of sprays were given at 10 days interval using knapsack sprayer at the rate of 2 ml l⁻¹ of water. Leaf samples were collected at different days (0, 1,3,5,7,10,15,20, 30 and 35 days) until it reached below detection level (BDL). Recoveries of chlorpyrifos 20EC were 102, 118 and 108% from the samples fortified at 0.05, 0.25, and 0.5 µg g⁻¹ respectively. Initial deposits were found to be 12.60 µg g⁻¹ and 26.14 µg g⁻¹ in recommended and double the recommended dose. More than 80% of initial deposit was dissipated within three days both in recommended and double the recommended dose and reached BDL by 30th and 35th day, respectively. Half life of chlorpyrifos 20EC residues were 4.15 and 4.4 days in recommended and double the recommended dose, respectively.

Key words: Curryleaf, Chlorpyrifos 20EC, Residues, QuEChERS method

Curry leaf (*Murraya koenigii* L. Spreng) is a leaf spice that belongs to a family Rutaceae known for its characteristic aroma and medicinal value. It is a rich source of vitamin A, vitamin C, iron and calcium (Vandhana *et al.*, 2012). The various pharmacological activities of the plant include, activity on heart, anti diabetic, cholesterol reducing property, anti ulcer activity, anti oxidative property (Arulselvan *et al.*, 2007), anti histaminic action and anti inflammatory action (Parmar *et al.*, 2010). It contains carbazole alkaloids that have anticarcinogenic effects (Akbar *et al.*, 1995) also.

Curryleaf is one of the major leaf spices exported from India. Many of the importing countries have imposed checks on import of curryleaf into their territory to monitor pesticide residue level (Pinyupa *et al.*, 2009). Citrus butterfly, psyllid bug, tortoise beetles, mealy bugs and scale insects are the major pests that attack curry leaves. For the control of these insect pests, organochlorines and organophosphate insecticides are widely used by farmers, even though these insecticides are not recommended. This unwanted and indiscriminate use of insecticides poses direct and indirect health hazards in human. There have been detectable levels of OC's and OP's and synthetic pyrethroids in curry leaves. MRLs (Maximum Residue Level) for most of the pesticide in use are not addressed by Codex Alimentarius Commission. With the view to estimate the residues of widely used insecticide, chlorpyrifos 20 EC and to assess MRLs, the present studies were undertaken mainly to overcome trade barriers.

Materials and Methods

Field experiment

The open field experiment was conducted from

September to November 2015 in Randomized Block Design (RBD) with three replications in farmer's field at Chinnamathampalayam, Coimbatore. Curry leaf (local cultivar Sengambu) was raised in 5 cent plots with a spacing of 1.2x1.5m². The crop was grown by following recommended agronomic practices. The commercial formulations of chlorpyrifos 20 EC was purchased locally. The first spraying was given one month after pruning using hand operated knapsack sprayer and subsequent application was made at 10 days interval with 500 L volume of spray fluid ha⁻¹. A control was also maintained. It was ensured that chlorpyrifos 20 EC was not used earlier in the experimental plot.

Recovery experiment

Recovery studies were carried out in order to establish the reliability of the method and to understand the efficiency of extraction and clean up steps employed for the present study. The untreated curry leaves (10 g) were spiked at three concentrations such as *viz.*, LOQ level (0.05), 5 x LOQ (0.25) and 10 x LOQ (0.5) µg g⁻¹ using analytical standard solution of chlorpyrifos 20 EC along with untreated control and replicated three times. The spiked samples were equilibrated and processed by adopting QuEChERS method (Sinha *et al.*, 2012). The residue of chlorpyrifos 20EC was estimated by GC coupled with Electron Capture Detector (GC-ECD)

Collection and processing of samples for residue analysis

Approximately 1 kg of curry leaf sample was collected randomly from each plot at 0 (one hour after spraying), 1, 3, 5, 7, 10, 15, 20, 30 and 35 days after the last application of chlorpyrifos 20 EC. All the collected samples were transported to the laboratory

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and processed immediately. All the processed samples were stored at -4° C before analysis.

Sample preparation

Curry leaves (1 kg) were chopped into small pieces, mixed thoroughly and sub samples were drawn by quartering method. The samples were homogenized in a blender for 2 min. The leaf samples from each plot were pooled, air dried and then passed through a 2 mm sieve and extracted immediately after sampling.

Extraction

A representative leaf sample of 10 g was transferred into a 50 ml centrifuge tube and mixed using a vortex for one min. after adding 20 ml of acetonitrile. Then, 4 g of anhydrous MgSO₄ and one g of NaCl were added and again shaken well by vortex, then centrifuged at 6000 rpm for 10 min.

Clean-up

After centrifuging, 6 ml of supernatant aliquot was transferred into a 15 ml centrifuge tube containing 100 mg PSA (Primary Secondary Amine), 600 mg anhydrous MgSO₄ and 100 mg graphitized carbon black(GCB). The mixture was vortexed for one min. and then centrifuged for 10 min, at 3000 rpm. The upper extract (4 ml) was transferred into a turbovap tube and concentrated to dryness under a gentle stream of nitrogen in a turbovap LV at 40° C. The final volume was reconstituted to about one ml using n-hexane and transferred into a 1.5 ml glass auto sampler vial for GC-ECD analysis.

Chemicals and reagents

The reference standards of chlorpyrifos 20 EC (98.2% purity) was purchased from M/S Sigma Aldrich, Bangalore, India. Acetonitrile, hexane (HPLC grade), sodium chloride and anhydrous magnesium sulfate (analytical grade) were purchased from M/S Merck (Mumbai, India). Primary Secondary Amine (PSA) (Bondesil 40 µm) and graphitized carbon black (GCB) were purchased from M/S Agilent technologies, USA.

Final determination of pesticide residues

GC- ECD

Determination of chlorpyrifos 20 EC residues was carried out by GC (Shimadzu 2010) equipped with electron capture detector (ECD). The operating temperatures were 250° C (injector), 280° C (detector) and oven temperature was programmed as follows: 160° C for 0 min., increased at the rate of 15° C/ min. to 280° C and hold for 10 mins. The Ultra high pure nitrogen was used as carrier gas at a constant flow rate of 0.95 ml min⁻¹. The injection volume was 2 µl with split ratio of 1: 2 with an auto sampler (Shimadzu ADL 20S) and an auto injector (AOC 20i). The retention time for chlorpyrifos 20 EC was 6.24 min at the above conditions.

Quantification of residues

The amount of residues was determined by comparing the sample peak area with the peak area of standard by using the formula

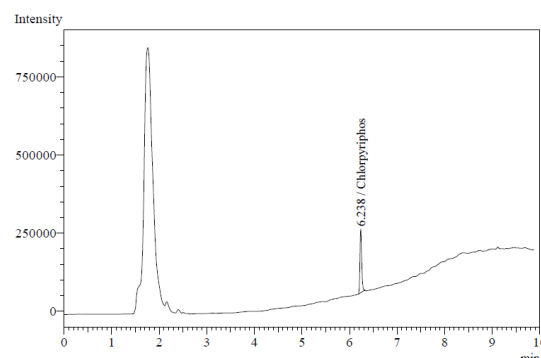


Fig 1. Chromatogram of chlorpyrifos 20 EC standard (0.1 µg g⁻¹)

$$\text{Residue } (\mu\text{g/g}) = \frac{A_s}{A_{\text{std}}} \times \frac{V_{\text{std}}}{W_s} \times \frac{V_s}{A_{\text{stdj}}}$$

where,

- A_s - peak area of the sample
- A_{std} - peak area of the standard
- V_{std} - concentration of the standard in ppm
- W_s - weight of the samples in g
- V_s - final volume of the sample in ml
- A_{sj} - injected volume of the sample in µl
- A_{stdj} - injected volume of the standard in µl

Recovery

The amount of recovery was determined by comparing the sample peak area with the peak area of standard (Fig 1) by using the formula

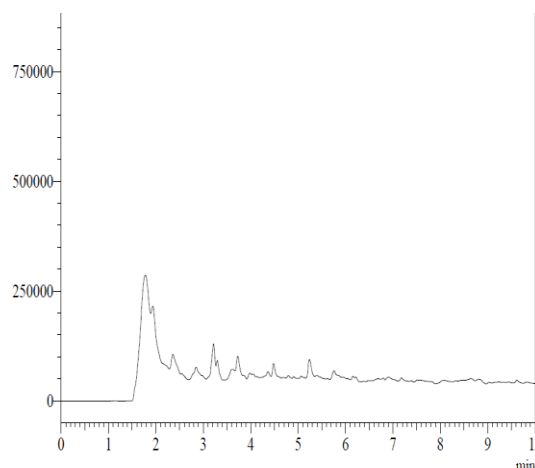


Fig.2. Chromatogram of control curry leaf sample

$$\text{Recovery } \% (\mu\text{g/g}) = \frac{A_s}{A_{\text{stdj}}} \times \frac{V_{\text{std}}}{W_s} \times \frac{V_s}{A_{\text{std}}} \times 100 / \text{fortification value}$$

where,

- A_s - peak area of the sample
- A_{std} - peak area of the standard

V_{std} – concentration of the standard in ppm

W_s = weight of the samples in g

V_s = final volume of the sample in ml

A_{si} – injected volume of the sample in μl

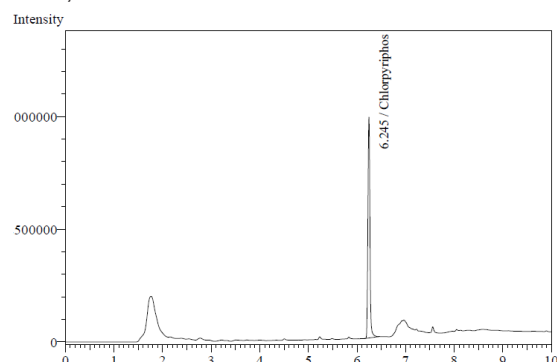


Fig. 3. Chromatogram of pesticide present in 0 day sample

Statistical analysis

The relative standard deviation (RSD) for mean recoveries is calculated

$$\text{RSD} = 100 \times \frac{S}{\bar{X}}$$

where

S = standard deviation

\bar{X} = arithmetic mean

Results and Discussion

The average recovery of chlorpyrifos 20EC fortified at 0.05, 0.25 and 0.5 $\mu\text{g g}^{-1}$ for curry leaf is shown in Table 1. For the amount fortified ranging from 0.05 to 0.5 $\mu\text{g g}^{-1}$ in curry leaf, chlorpyrifos 20 EC showed recovery values ranging from 102 to

108 per cent. The result showed that the analysis by GC gave good recoveries at different concentration indicating good performance of extraction, cleanup and chromatographic parameters.

Dissipation studies

Mean initial deposits of 12.60 $\mu\text{g g}^{-1}$ and 26.14 $\mu\text{g g}^{-1}$ of chlorpyrifos 20 EC were detected in samples, which dissipated to 1.31 $\mu\text{g g}^{-1}$ and 2.85 $\mu\text{g g}^{-1}$ by 5th day and below detection level by 30th and 35th days in recommended and double the recommended dose. More than 80% of initial deposit was dissipated within 3 days. Dissipation pattern along with regression equation and half-life values are given in Table 2 and 3.

Table 1. Recovery study of chlorpyrifos 20 EC on curryleaf at various fortification level

Spike level $\mu\text{g g}^{-1}$	Recovery %	RSD %
	Mean \pm SD	
0.05	102.59 \pm 4.36	4.25
0.25	118.37 \pm 2.10	1.77
0.5	108.44 \pm 2.12	5.12

SD-Standard deviation

RSD-Relative standard deviation

Regression equation indicates that increase in 1 unit of time will lead to 3.831 units and 4.141 units of residues in curry leaves at recommended and double the recommended doses. Half life values were found to be 4.15 and 4.4 days in recommended and double the recommended dose, respectively. Chromatogram of control curry leaf samples and 0 day samples is shown in Fig 2 and 3. Similar pattern of chlorpyrifos dissipation was observed in curry leaves where, more than 50 per cent dissipated within

Table 2. Dissipation of chlorpyrifos 20 EC recommended (X dose) at different intervals (days)

Days after spraying	Residue in $\mu\text{g/g}$				
	Recommended dose (X dose) (300 g a.i. ha ⁻¹)				
	R1	R2	R3	Mean \pm SD	Dissipation %
0 (1hr after spraying)	13.00	12.12	12.75	12.60 \pm 0.45	-
1	11.39	10.61	11.71	11.06 \pm 0.40	12.43
3	1.51	1.41	1.48	1.47 \pm 0.06	88.36
5	1.34	1.25	1.32	1.31 \pm 0.05	89.62
7	1.26	1.18	1.24	1.23 \pm 0.04	90.26
10	0.86	0.80	0.84	0.84 \pm 0.03	93.34
15	0.51	0.48	0.50	0.50 \pm 0.02	96.04
20	0.33	0.30	0.32	0.32 \pm 0.02	97.46
30	BDL	BDL	BDL	BDL	100

BDL – Below detectable level,

Regression equation $Y = -0.072x + 3.75$

$R^2 = 0.770$

Half-life = 4.15 days

3 days with half-life of 1.5 days and reached below detectable level on 15th day. The difference in half-life might be due to climatic conditions. Walia *et al.* (1988)

reported that chlorpyrifos sprayed on leaf surface produced new products due to photochlorination, oxidation and hydrolytic process under the influence

of sunlight thus, reducing photodegradation of chlorpyrifos on leaf surface, which made the pesticide to persist. Also chlorpyrifos has high

hydrophobicity, the pesticide would readily enter into the trans laminar penetration parts from leaf surface resulting in high residue level.

Table 3. Dissipation of chlorpyrifos 20 EC double the recommended dose (2X dose) at different intervals

Days	Residue in µg/g double the recommended dose(2x dose) (600 g a.i. ha ⁻¹)				Dissipation %
	R1	R2	R3	Mean ±SD	
0(1hr after spraying)	26.92	25.09	26.40	26.14±0.94	-
1	11.39	10.61	11.17	11.06±0.40	8.76
3	3.34	3.12	3.28	3.25±0.11	87.56
5	2.93	2.73	2.87	2.85±0.10	89.09
7	2.67	2.49	2.62	2.60±0.07	90.00
10	1.75	1.1.63	1.71	1.70±0.06	93.49
15	1.20	1.22	1.14	1.19±0.04	95.44
20	0.84	0.86	0.80	0.84±0.03	96.78
30	0.21	0.20	0.21	0.21±0.02	99.19
35	BDL	BDL	BDL	BDL	100

BDL – Below detectable level, Regression equation $Y = -0.068x + 4.073$ $R^2 = 0.740$ Half-life = 4.40 days

This stability and effectiveness made chlorpyrifos one of the most popular pesticides, but its persistence had raised environmental concern. Meng-Xiao Lu *et al.* (2014) reported that the initially deposited chlorpyrifos amount depended upon the surface area of the foliages with which the pesticide was sprayed. The foliages of pepper plant, celery and asparagus were overlapping to maximize the effect on foliar surface area, so the concentration of initial deposition is high. The concentration of initial deposition is directly proportional to the foliar surface area (Gaskin *et al.*, 2005)

Conclusion

Initial deposit of chlorpyrifos 20 EC was found to be 12.60 and 26.14 in recommended and double the recommended dose and half-lives were 4.15 and 4.4 days, respectively. Results showed that chlorpyrifos 20EC had dissipated by 30 and 35 days in recommended and double the recommended dose. Maximum residue limit of chlorpyrifos 20 EC on curry leaf is not fixed by codex alimentarius commission as on date.

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