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# Dissipation and Persistence of Chlorpyriphos 20EC in / on Curry Leaf

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**Dissipation of chlorpyriphos 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, 30and 35 days) until it reached below detection level (BDL). Recoveries of chlorpyriphos 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 30$^{th}$ and 35$^{th}$ day, respectively. Half life of chlorpyriphos 20EC residues were 4.15 and 4.4 days in recommended and double the recommended dose, respectively.**

**Key words:** Curryleaf, Chlorpyriphos 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 pesiticide 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, eventhough 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, chlorpyriphos 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 1.2x1.5$m^2$. The crop was grown by following recommended agronomic practices. The commercial formulations of chlorpyriphos 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 chlorpyriphos 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 × LOQ (0.25) and 10 × LOQ (0.5) µg $g^{-1}$ using analytical standard solution of chlorpyriphos 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 chlorpyriphos 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 chlorpyriphos 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 $MgSO_4$ 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 $MgSO_4$ 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 chlorpyriphos 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 chlorpyriphos 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 chlorpyriphos 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

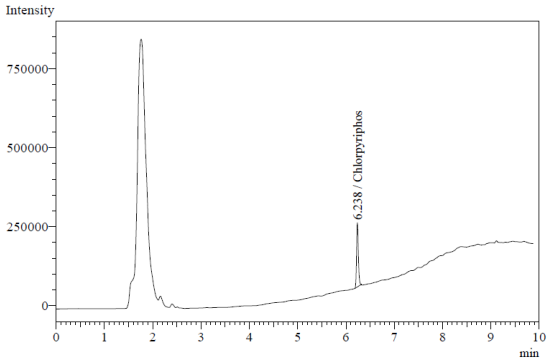


**Fig 1.Chromatogram of chlorpyriphos 20 EC standard(0.1µg $g^{-1}$)**

Residue (µg/g) = $A_s/A_{std}$ X $V_{std}/W_s$X $V_s/ A_{sj}$ X $A_{stdj}$

where,

$A_s$ - peak area of the sample

$A_{srd}$ - peak area of the standard

$V_{srd}$ - 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

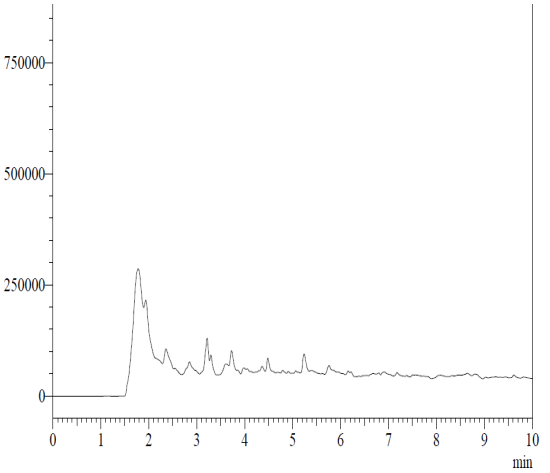


**Fig.2. Chromatagram of control curry leaf sample**

Recovery % (µg/g) = $A_s/A_{std}$ X $V_{std}/W_s$X $V_s/ A_{sj}$ X $A_{stdj}$X 100/fortification value

where,

$A_s$ – peak area of the sample

$A_{srd}$ – peak area of the standard

$V_{srd}$ – 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

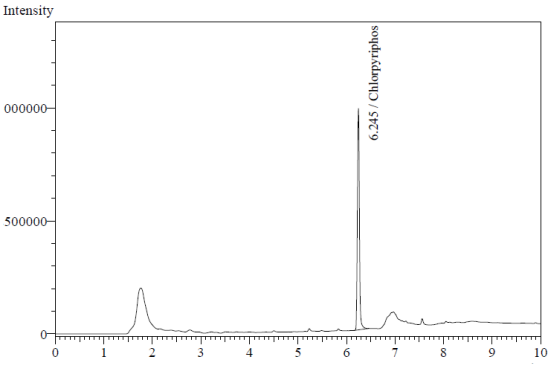


**Fig. 3. Chromatogram of pesticide present in 0 day sample**

***Statistical analysis***

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

RSD = 100 X S xi

where

S = standard deviation

Xi = arithmetic mean

**Results and Discussion**

The average recovery of chlorpyriphos 20EC fortified at 0.05, 0.25 and 0.5µg $g^{-1}$ for curry leaf is shown in Table 1. For the amount fortified ranging from 0.05 to 0.5 µg $g^{-1}$ in curryleaf, chlorpyriphos 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 µg $g^{-1}$ and 26.14 µg $g^{1}$ of chlorpyriphos 20 EC were detected in samples, which dissipated to 1.31µg $g^{-1}$ and 2.85 µg $g^{-1}$ by 5$^{th}$ day and below detection level by 30$^{th}$ and 35$^{th}$ 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 chlorpyriphos 20 EC on curryleaf at various fortification level**

| Spike level µg $g^{-1}$ | Recovery % Mean ± SD | RSD % |
|---|---|---|
| 0.05 | 102.59±4.36 | 4.25 |
| 0.25 | 118.37±2.10 | 1.77 |
| 0.5 | 108.44±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 chlorpyriphos dissipation was observed in curry leaves where, more than 50 per cent dissipated within 3 days with half-life of 1.5 days and reached below detectable level on 15$^{th}$ day. The difference in half-life might be due to climatic conditions. Walia *et al*. (1988) reported that chlorpyriphos sprayed on leaf surface produced new products due to photochlorination, oxidation and hydrolytic process under the influence

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

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

of sunlight thus, reducing photodegradation of chlorpyriphos on leaf surface, which made the pesticide to persist. Also chlorpyriphos 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 chlorpyriphos 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. $ha^{-1}$) | | | | |
|---|---|---|---|---|---|
| | R1 | R2 | R3 | Mean ±SD | Dissipation % |
| 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 chlorpyriphos one of the most popular pesticides, but it's persistence had raised environmental concern. Meng-Xiao Lu *et al.* (2014*)* reported that the initially deposited chlorpyriphos 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 chlorpyriphos 20 EC was found to be 12.60 and 26.14 in recommended and double the recommended dose and half-lives were 4.15and 4.4 days, respectively. Results showed that chlorpyriphos 20EC had dissipated by 30 and 35 days in recommended and double the recommended dose. Maximum residue limit of chlorpyriphos 20 EC on curry leaf is not fixed by codex alimentarius commission as on date.

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