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## Dissipation behaviour, quantification and risk assessment of Chlorpyrifos in green pea by gas chromatograph

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**Abstract**

The present study was conducted to evaluate the persistence, half-life, dissipation behaviour and risk assessment of Chlorpyrifos on green pea by gas chromatography-electron capture detector. Chlorpyrifos 20 EC at 300g.a.i.ha<sup>-1</sup> was applied during fruiting stage followed by another application at 10 day interval. The samples were extracted by using quick, easy, cheap, effective, rugged, and safe (QuEChERS) method. Herein we report a modified accurate and cost-effective gas chromatography method for the determination of average deposits of Chlorpyrifos on green pea. The initial deposits were found to be 1.37 mg kg<sup>-1</sup> and reached below limit of determination after 15 days at recommended dosage with half-life period of 1.77 days. For risk assessment studies, a waiting period of 15 days at the recommended dosages is suggested safe for the consumption of green pea. The developed method is simple, sensitive, selective and repeatable and can be extended for Chlorpyrifos based standardisation of herbal formulations containing green pea and its use in pesticide industries.

**Keywords:** Chlorpyrifos, green pea, gas chromatography, quantification, pesticides

**Introduction**

Chlorpyrifos (C<sub>9</sub>H<sub>11</sub>Cl<sub>3</sub>NO<sub>3</sub>PS) *O,O*-Diethyl *O*-3,5,6-tri chloro pyridinyl phosphorothioate is an organophosphate pesticide (Fig. 1) used to kill a number of pests including insects and worms. It is used on crops, animals, and buildings (Rathod, 2017) [16]. Chlorpyrifos was first registered for use in the United States in 1965 for control of foliage and soil-born insects (U.S. EPA 2012) [21]. In agriculture, it is one of the most widely used organophosphate insecticides in the United States, and before being phased out for residential use was one of the most used residential insecticides (WHO 2009) [22]. Chlorpyrifos is considered moderately hazardous to humans by the World Health Organization. Exposure during pregnancy may harm the mental development of children and its home use was banned in 2001 in the U.S. [4] it acts on the nervous system of insects by inhibiting acetyl cholinesterase. The mild poisoning of Chlorpyrifos can result in eye watering, increased saliva, sweating, nausea and headache. Intermediate exposure may lead to muscle spasms or weakness, vomiting or diarrhea and impaired vision. Symptoms of severe poisoning include seizures, unconsciousness, paralysis, and suffocation from lung failure (Christensen, *et al.*, 2009) [5].

Green pea (Fabaceae family) is one of the ancient cultivated herbaceous vine originated in the sub-Himalayan plains of northwest India and grown for their delicious, nutritious green seeds. It is one of the major commercial crops grown all over the temperate and semi-tropical regions. Today, this versatile legume is one of the most nutritious vegetables rich in health promoting secondary metabolites ( $\beta$ -sitosterol), minerals (Ca, Cu, Fe, Mg, Mn, Se, Zn), vitamins (A, B, C, E, K) and antioxidants (carotenes, lutein, and zeaxanthin) (Dahl, *et al.*, 2012) [9].

QuEChERS, an inexpensive and fast method named quick, easy, cheap, effective, rugged, and safe was first introduced (Anastassiades, *et al.*, 2003) [2]. for monitoring of pesticide residues in fruits and vegetables. The method has been used across the world in many studies for pesticide residue analysis in different vegetable samples (Aysal, *et al.*, 2007) [3] in the recent times as a result of high recovery of pesticides with large polarity and volatility range and the need for small amounts of organic solvents eliminating the use of chlorinated solvents. Dissipation of pendimethalin in the soil of field Pea crop and terminal residues in green and mature field peas has been reported by (Shobha & Sondhia 2013) [17]. Dissipation behaviour of ethion and quinalphos on green pea also has reported in literature and herein we report dissipation behaviour of Chlorpyrifos on green pea by applying modified

QuEChERS method for sample preparation followed by quantification and estimation of residues by gas chromatography. Additionally, the risk assessment of Chlorpyrifos residues on human health is also reported.

## Materials and methods

### Reagents and chemicals

The technical grade analytical standard of Chlorpyrifos (purity 99.3 %) was procured from M/s Sigma Aldrich, India. The formulation (Chlorpyrifos 20EC) was obtained from M/s Premier Sales Agency, Srinagar, Jammu & Kashmir, India. Analysis of hexane extract of the formulation showed only Chlorpyrifos and none of its metabolic product. Solvents and reagents like acetonitrile, acetone, hexane, sodium chloride (NaCl), (ASC reagent grade  $\geq 99.9\%$ ) and analytical grade activated anhydrous  $MgSO_4$  were obtained from Merck, Darmstadt, Germany. Sodium sulphate ( $Na_2SO_4$ ) anhydrous (AR grade) was obtained from S. D. Fine Chemicals, Mumbai. Primary Secondary Amine (PSA) Sorbent and activated graphitic carbon black (GCB, 400mesh) were obtained from Agilent Technologies, Bangalore, India. All solvents were redistilled prior to use and the suitability of the solvents and other chemicals were ensured by running reagent blanks before actual analysis.

### Preparation of standard solution

Standard stock solutions of Chlorpyrifos ( $1\text{ mg mL}^{-1}$ ) were prepared in HPLC grade hexane for GC-ECD analysis. For calibration curve different concentrations ( $1.5, 1.0, 0.5, 0.10, 0.05,$  and  $0.01\ \mu\text{g mL}^{-1}$ ) were prepared with appropriate dilution of the stock solution. The solutions were filtered through  $0.45\ \mu\text{m}$  PTFE filter before analysis. All standard stock solutions and working standard solutions were stored at  $4^\circ\text{C}$  before use.

### Instruments

The cleaned extract were analysed on GC-Varian 450 equipped with capillary column using  $Ni^{63}$  electron capture detector (ECD). The separation of pesticides was done on a Capillary column: CPSIL-8CB having 30 meter length,  $0.25\text{ mm}$  internal diameter and  $0.25\ \mu\text{m}$  film thickness.

### Field experiment

The experiment was done on Green Pea (Arkel) which was raised during summer 2017 at SKUAST main Campus, Shalimar, Srinagar (J&K) following recommended agronomic practices (Derksen, *et al.*, 1993) [6]. The plot was arranged in a randomized block design (RBD), in which the size of each plot was  $84\text{sqm}$ . There were three replications for each treatment (i.e. control and recommended dosage). The first application of Chlorpyrifos 20EC at  $300\text{ g a.i.ha}^{-1}$  was made at fruiting stage followed by another application at 10-day interval. Pesticide was sprayed as foliar application with foot sprayer fitted with hollow cone nozzle. About  $1.0\text{ kg}$  fruits of marketable size were collected randomly at 0 (2 h after spray), 1, 3, 5, 7, 10 and 15 days after the second application of the pesticide. The green pea fruits were collected from each plot separately, packed in polyethylene bags, and brought to the laboratory for processing. Samples were extracted and cleaned up immediately after sampling. During the period of experiment (May 18, 2017 to June 13, 2017), the total rainfall ( $85.7\text{ mm}$ ) and the meteorological data are presented in Fig 2.

### Extraction and Clean up with QuEChERS method

For the determination of Chlorpyrifos residues, the samples of

green pea from day 0, 1, 3, 5, 7, 10 and 15 were processed by following QuEChERS method. Green pea pod ( $1.0\text{kg}$ ) was chopped and grinded in a mixer grinder for 3 min. A representative sample of  $15\text{ g}$  was weighed into a  $50\text{-mL}$  centrifuge tube and then  $30\text{-mL}$  HPLC grade acetonitrile was dispensed into it. The sample was homogenized using high speed homogenizer (Pro Scientific) for 2-3 min at  $14,000\text{-}15,000\text{ rpm}$ . For good phase separation sodium chloride (NaCl)  $3.0\pm 0.1\text{ g}$  was added to the sample. The contents were centrifuged at  $2500\text{-}3000\text{ rpm}$  for 3 min. An aliquot of  $18\text{-mL}$  acetonitrile layer was transferred into another  $50\text{-mL}$  centrifuge tube containing  $9.0\pm 0.1\text{ g}$  sodium sulphate ( $Na_2SO_4$ ). The acetonitrile extract was subjected to clean up by dispersive solid phase extraction (DSPE). An aliquot of  $1\text{ mL}$  acetonitrile was taken in a test tube containing  $1.15\text{ g}$   $MgSO_4$  and  $0.4\text{g}$  primary secondary amine (PSA) and vortexed for  $30.0\text{ sec}$  followed by centrifugation at  $3000\text{rpm}$  for  $5.0\text{ min}$ . Six-millilitre acetonitrile aliquot was evaporated to dryness using low-volume evaporator at  $35^\circ\text{C}$ . Final volume was reconstituted with  $3\text{ mL}$  n-hexane.

### GC analysis

Gas chromatography (GC) was used for the identification and estimation of Chlorpyrifos residues in green pea. A capillary column (CPSIL-8CB,  $0.25\ \mu\text{m}$  film thickness  $\times 0.25\text{mm I.d} \times 30\text{m}$  length) was used. Before analysis, the column was prepared with several injections of standard solution of Chlorpyrifos till a reliable response was obtained. The nitrogen was used as make up gas with flow rate of  $35\text{ mL min}^{-1}$ . The temperatures for injector and detector were maintained at  $240,$  and  $300^\circ\text{C}$  respectively. The temperature of the oven was programmed from an initial value of  $170^\circ\text{C}$  for 2 min, ramped to  $230^\circ\text{C}$  at  $6^\circ\text{C min}^{-1}$  for 5 min, and to  $260^\circ\text{C}$  at  $10^\circ\text{C min}^{-1}$  for 5min, and was raised to  $270^\circ\text{C}$  at  $10^\circ\text{C min}^{-1}$  for 5 min. Suitable aliquots ( $1\ \mu\text{L}$ ) of the cleaned samples were then injected into electron capture mode of the detector. Under these operating conditions, the retention time of Chlorpyrifos was found to be  $10.92\text{ min}$  (Fig. 5). The run time for the developed method has been fixed for 31 minutes. The limit of quantification (LOQ) of Chlorpyrifos was found to be  $0.05\text{ mg kg}^{-1}$ . Retention times and peak height/peak areas of standards were compared with those of unknown or spiked samples run under identical conditions for estimation of Chlorpyrifos residues.

## Results and discussion

### Method Validation

Specificity, Recovery studies, linearity, LOD, LOQ, Ruggedness and Precision were performed at different levels by following the SANCO guidelines (SANCO (2013) [18]) to examine the efficiency of extraction, reliability of GC method and clean up, by analyzing reference compound and the analyses.

### Specificity

The specificity of the method was obtained by analyzing standards and sample. The species giving rise to the signal used for quantification shows that the detected peak is solely due to the analyte, not another compound. The absence of any interfering peak indicated that the method is specific.

### Accuracy

Recovery studies were performed by addition of known amounts of pesticide to extract solution at the three spiking levels to verify the accuracy of the method. Untreated samples

of green pea after homogenization were spiked by addition of appropriate volumes of pesticide standard solution (0.05, 0.1, and 0.5 mg kg<sup>-1</sup>) respectively, and were left for 1 h (equilibration times) and then prepared according to the procedures described in the "Extraction and cleanup with QuEChERS method" section. The accuracy was then calculated from the test results as the percentage of recovery and the results have been summed up in Table 1. Per cent recoveries of Chlorpyrifos in green pea were found to be Consistent (Dar *et al.*, 2015; Dar *et al.*, 2014; Dar, *et al* 2016; Khan *et al.*, 2013) [7, 8, 10, 14]. (Equation 1) and more than 85% (Table 1) that indicates the developed method is accurate.

The recovery (%) was calculated by the equation (1)

Recovery (%) = (found amount-original amount)/spiked amount × 100 (1)

### Linearity

Six point calibration curve has been constructed to evaluate the linearity of the developed method with three replicates over a concentration range of 0.05 to 2.0 µg mL<sup>-1</sup> by calculating the linear regression and squared correlation coefficient ( $R^2$ ). The lowest concentration level in the calibration curve was established as a limit of detection. GC-Galaxie software was used to develop the calibration curve by plotting peak area versus concentrations (n=6) using linear regression analysis. Good correlation coefficient was observed which showed goodness of fit within the test range of 0.998 (Fig. 3).

### Limit of detection and Limit of quantification

The LOD and LOQ have been calculated by following the SANCO guidelines. The LOD and LOQ were evaluated as the signal-to-noise ratio (S/N) of 3:1 and 10:1 for the pesticide, respectively. The limit of detection (LOD) was defined as the lowest concentration of the standard which could be detected but not necessarily quantified. The limit of quantification (LOQ) was defined as the lowest concentration of the analyte in a sample that could be quantified with acceptable precision and accuracy. LOD and LOQ of Chlorpyrifos were found to be 0.01 and 0.05 µg mL<sup>-1</sup> respectively.

### Ruggedness

The ruggedness of an analytical method was obtained by analysis of same sample under a variety of normal test conditions i.e., small variation in mobile phase, flow rate of mobile phase, temperature, relative humidity, reagents, and different analysts etc. The effect on GC analysis under these conditions was found less than 20% (≤1.21%RSD) indicating the ruggedness of the method.

### Precision

The precision of each compound was evaluated at five levels (0.05, 0.1, 0.5, 1 and 1.5 mg kg<sup>-1</sup>). Six samples at each level for each matrix were analyzed in different days (intra and inter-day). Precision of the method was expressed as the percentage of relative standard deviation and it should be <20% (3.21% RSD).

### Persistence of Chlorpyrifos in green pea fruit

Table 2 presents the overall results of analysis of green pea fruit samples for estimation of Chlorpyrifos residues. The average initial deposits of Chlorpyrifos on green pea fruit were found to be 1.37 mg kg<sup>-1</sup>, after two applications at 10-day interval of Chlorpyrifos 20EC at 300 g.a.i. ha<sup>-1</sup>. The Maximum Residue Levels of Chlorpyrifos on green pea as

fixed at the national level by Food Safety & Standard Authority of India (0.2 ppm) and the international level by Codex (0.01 ppm) So, the residue levels compared with the same values and reached below limit of detection (LOD) of 0.01 mg kg<sup>-1</sup> after 15<sup>th</sup> days at recommended dosage. The above findings revealed that higher rate of application of Chlorpyrifos resulted in higher initial deposits. The residues of Chlorpyrifos decreased over the time and dissipation pattern of green pea are shown in (Fig. 4). Based on the results, the maximum concentration of Chlorpyrifos residues in green pea samples was found two hour after pesticide application, and its concentration decreased with increasing the storage time. This result is in accordance with other studies on the persistence of Chlorpyrifos residues, and the same trend has been observed for Ethion and Quinalphos in green pea (Dar *et al.*, 2018; Jan *et al.*, 2018; Leili *et al.*, 2016) [11, 13, 15].

### Degradation kinetics and half-life of Chlorpyrifos

The degradation kinetics of the Chlorpyrifos in green pea was determined by comparing residue concentration against time, and the results are presented in table 2. The persistence of Chlorpyrifos was expressed in terms of half-life ( $T_{1/2}$ ) or DT<sub>50</sub>. The dissipation of Chlorpyrifos on green pea followed first-order kinetics. Half-life ( $T_{1/2}$ ) of Chlorpyrifos calculated as per Hoskins (Hoskins, W.1961) [12] was observed to be 1.77 days, when applied at 300 G.A.I. ha<sup>-1</sup>. H. Fang *et al.* also observed that Chlorpyrifos in the mineral salts medium of pH 7.0 at 25 °C follow first-order kinetics. The residues of Chlorpyrifos on green pea dissipated BDL on 7th day following first order kinetics with half-life ( $t_{1/2}$ ) period of 2.83 days when applied 300 G.A.I. ha<sup>-1</sup> (Sushil Ahlawat 2017) [19].

### Risk assessment of Chlorpyrifos in green pea fruit

When the use of pesticides on food crops exceed maximum residue limit (MRL) it may leads to unwanted residues, which may constitute barriers to exporters and domestic consumptions. Theoretical maximum residue contributions (TMRC) were calculated from the residues (average and maximum) of Chlorpyrifos observed at different intervals of time (Table 4) and then compared with maximum permissible intake (MPI) to evaluate the risk to the consumer. The prescribed Chlorpyrifos acceptable daily intake is 0.01 mg kg<sup>-1</sup> body weight/day (Sharma KK 2007) [20]. Maximum permissible intake was calculated by multiplying acceptable daily intake with the weight of average person (55 kg) and was found to be 550 µg person<sup>-1</sup> day<sup>-1</sup>. Taking average consumption of green pea fruit as 80g per day (Ahmad *et al.*, 2016) [1] maximum and average residues of Chlorpyrifos on green pea were used for the calculation of theoretical maximum residue contributions values. Theoretical maximum residue contributions values were found to be 109.6 and 112.0 µg person<sup>-1</sup> day<sup>-1</sup> (Table 3) for average and maximum Chlorpyrifos residues respectively, when applied at 300 g.a.i. ha<sup>-1</sup>. Although it was observed that theoretical maximum residue contributions values reached below Maximum permissible intake on 0 day after treatment of green pea fruit samples with Chlorpyrifos at given dosages, the residue half-life values 1.77 days and the residues persisted on 15th day of application. Hence, a waiting period of 15 days before consuming green pea fruit was suggested to further reduce the risk. Therefore, these studies suggested that there is no threat to human beings if they consume green pea fruit treated with formulations of Chlorpyrifos after 15th day of application.

## Conclusions

This study investigated the dissipation, risk assessment, half-life and chemo biological standardisation of Chlorpyrifos by gas chromatography. We use the pesticides to have the higher and insect free yield. But the overdoses of pesticides make the residue problem, which might pollute our food and environment. Continuous and haphazard utilization of pesticides leads to accumulation of residues in consumable vegetables. The continuous ingestion of these residues, though in minute quantities, can result in their accretion in the body causing undesirable effects on human well-being. Therefore residue level of Chlorpyrifos reached below detection limit on 15<sup>th</sup> day after treatment with Chlorpyrifos at recommended dosages. Therefore a waiting period of 15 day is suggested to reduce the risk before consumption of green pea fruit samples. These results would be helpful for safe use of Chlorpyrifos on green pea and to prevent any health problem to consumers. Since the developed method is simple, sensitive, selective and repeatable it can be extended for Chlorpyrifos based standardisation of herbal formulations

containing green pea and its use in pesticide industries.

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## Conflict of interest

The authors have declared no conflict of interest.

**Table 1:** Recovery studies of Chlorpyrifos on green pea

Level of fortification (mg kg <sup>-1</sup> )	Recovery (%)
0.05	86.2
0.1	83.2
0.5	98.6

**Table 2:** Residues of Chlorpyrifos (mg kg<sup>-1</sup>) on green pea at different time intervals after the application of Chlorpyrifos 20EC at 300 g.a.i. ha<sup>-1</sup>

Chlorpyrifos 20EC@300 g.a.i.ha <sup>-1</sup>						
Days after application	Replicates	Mean	SD	%RSD	% Dissipation	Half-life (T <sub>1/2</sub> )
Before application	BDL	BDL	BDL	-----	-----	
0	1.40 1.36 1.37	1.37	0.020	1.45	-----	
1	0.74 0.63 0.55	0.64	0.095	14.84	53.28	
3	0.17 0.19 0.14	0.16	0.025	15.62	88.32	
5	0.15 0.12 0.16	0.14	0.020	14.28	89.78	1.77days
7	0.09 0.06 0.08	0.07	0.015	21.42	94.89	
10	0.02 0.02 0.02	0.02	0.0	0.0	98.54	
15	BDL	BDL	BDL	BDL	100.00	

SD: standard Deviation, RSD: Relative standard deviation  
BDL: Below determination limit of 0.01 mg kg<sup>-1</sup>

**Table 3** Maximum permissible intake (MPI) and theoretical maximum residue contribution (TMRC) of Chlorpyrifos in green pea fruits

Interval day	<sup>a</sup> MPI	Chlorpyrifos 20EC@300 g.a.i.ha <sup>-1</sup>			
		<sup>b</sup> Average residues in green pea fruits	<sup>a</sup> TMRC	<sup>b</sup> Maximum residues in green pea fruits	<sup>a</sup> TMRC
0	550	1.37	109.6	1.40	112
1	550	0.64	51.2	0.74	59.2
3	550	0.16	12.8	0.19	15.2
5	550	0.14	11.2	0.16	12.8
7	550	0.07	5.6	0.09	7.2
10	550	0.02	1.6	0.02	1.6
15	550	BDL	BDL	BDL	BDL

MPI: maximum permissible intake, TMRC: theoretical maximum residue contribution, BDL: below detection limit. a: (μg person<sup>-1</sup> day<sup>-1</sup>) b: (mg kg<sup>-1</sup>)

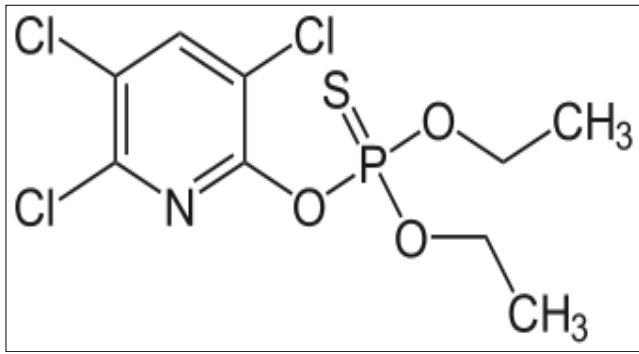


Fig 1: Structure of Chlorpyrifos

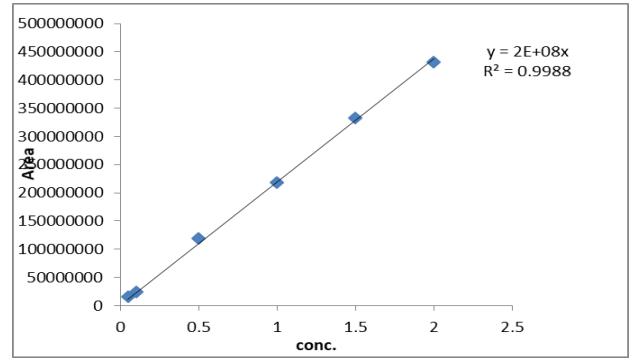


Fig 3: Calibration curve

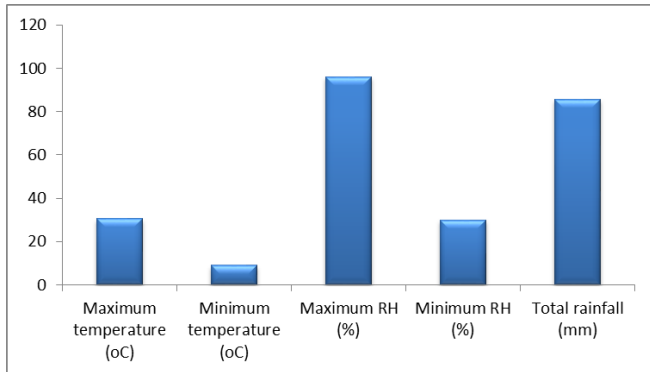


Fig 2: Meteorological data from May 18, 2017 to June 13, 2017

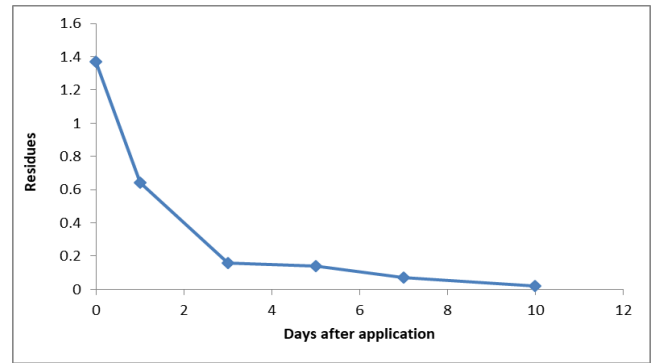


Fig 4: Dissipation pattern of Chlorpyrifos residues in/on green pea.

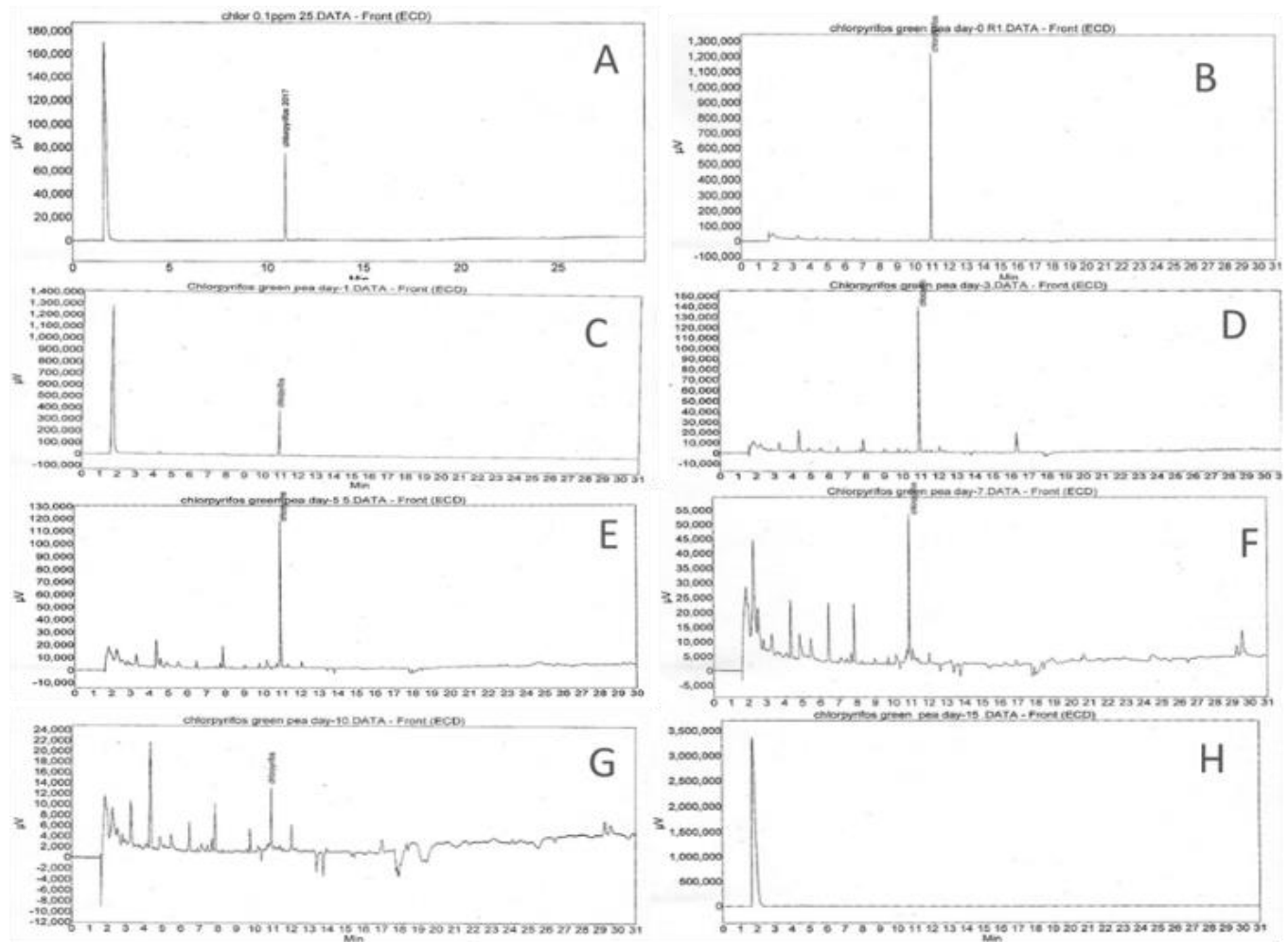


Fig 5: Segment [A]: GC chromatogram of Chlorpyrifos Standard (RT=11.92), Segment [B]: Day-0 Sample of green pea. Segment [C]: Day-1 Sample of green pea. Segment [D]: Day-3 Sample of green pea. Segment [E]: Day-5 Sample of green pea. Segment [F]: Day-7 Sample of green pea. Segment [G]: Day-10 Sample of green pea. Segment [H]: Day-15 Sample of green pea.

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